Hormonal Regulation of Growth in a Lizard, Anolis Carolinensis.

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ABSTRACT

Anolis appears to be "functionally hypophysectomized" when exposed to short photoperiods. Experiments were designed to determine whether daily injections of hormones could stimulate growth in anoles "functionally hypophysectomized" by exposure to six hours of light per day. Injections of either 10 mg./kg./day of bovine growth hormone or 7 mg./kg./day of desiccated thyroid stimulated growth due to increased tissue protein. Optimal growth occurred in animals injected with a combination of 10 mg./kg./day of growth hormone, 7 mg./kg./day of desiccated thyroid, and 25 mg./kg./day of insulin. Insulin alone did not stimulate growth. Unlike exposure to long photoperiods which stimulate growth with increased calorie consumption, hormone injections stimulated growth without augmentation of appetite. Growth of hormone injected animals appeared to result from more efficient utilization of food.

On the basis of nitrogen excretion rates during fasting at 28°C., catabolism of tissue proteins supplied 62% of standard metabolic requirements in spring and early summer but supplied only 20% in winter. Daily injections of growth hormone did not affect fasting nitrogen excretion. Glucose feeding "spared" 0.6 mg. tissue protein/mg. glucose fed.
The rate of catabolism of dietary protein also appeared to vary seasonally. Anoles were fed single high protein meals adequate in calories for six days. In spring the nitrogen excreted in three days after feeding was equivalent to protein fed. In autumn nitrogen excreted was equivalent to only 60% of protein fed. Nitrogen excretion of fed anoles injected daily with growth hormone was 25% greater than that of non-injected anoles. Nitrogen metabolism is active in spring and summer when dietary protein is utilized as an energy source and in growth processes. Nitrogen metabolism is decreased in winter when food consumption and growth processes are minimal, and maintenance calories are obtained from catabolism of stored fats and carbohydrates.

Growth processes cannot be fully understood without knowledge of the control of carbohydrate metabolism. In an attempt to obtain further evidence on basic factors responsible for observed seasonal differences in gross tissue composition, the manner in which anoles disposed of 40 mg. of glucose was determined at various seasons. In autumn tissue glycogen was stable and at a maximum concentration. Forty-eight hours after injection glucose appeared chiefly in liver glycogen. In winter carbohydrate was mobilized to extra-hepatic tissues where glucose utilization proceeded rapidly. In spring and summer when tissue glycogen was labile, injected glucose was probably rapidly oxidized.

Anolis was sensitive to bovine glucagon and insensitive to bovine insulin. Injection of 1 mg./kg. of glucagon abolished the hypoglycemic effect of 25 mg./kg. of insulin. Glucagon and insulin appear to act synergistically in the transportation of glucose from liver to muscle as occurs in winter. The hyperglycemic response to glucagon correlated with initial liver glycogen. Growth hormone caused a
hyperglycemia but, contrary to findings in other species, led to an 18% decrease in extra-hepatic glycogen. Tissue carbohydrate in "functionally hypophysectomized" anoles injected with a combination of growth hormone, desiccated thyroid, and insulin was low as in anoles during spring and summer.

Seasonal differences in the physiological state of Anolis appear to be controlled by cyclic changes in activities of its endocrine organs. Growth occurs only when endocrine secretions are in favorable balance.
INTRODUCTION

The term growth is applied to a myriad of phenomena which has as a common denominator an increase in mass (Thompson, 1917). In biological systems growth implies an increase in protoplasm or, in particular, an increase in protein of an organ or organ system. An important characteristic of biological growth is that it is limited; that is, for each species there is a maximum size for the organism as a whole and a proportional maximum size for each organ. This limitation imposed upon biological growth implies the existence of some regulatory mechanisms controlling growth.

Since the advent of endocrinology a plethora of data have accumulated relative to the hormonal regulation of growth in animals. The role of hypophyseal hormones of vertebrates in regulating growth of organs such as the thyroid (Adams, 1946; Albert, 1949; Sonenberg, 1958), adrenals (White, 1944; Li and Evans, 1948; Hays and White, 1954), primary sex organs (Fevold, 1944; Levin, 1944; Loraine, 1956), and mammary glands (White, 1949; Lyons, et al., 1958) is well documented. Secretions of the primary sex organs further regulate growth of secondary sex organs (Dorfman and Shipley, 1956; Engel, 1959). More recent data give evidence of growth regulatory effects of the pancreatic hormone insulin on the liver (Salter and Best, 1953). These are examples of hormonal regulation of growth of internal organs as distinguished from a change in body
size due to an increase in muscle mass and bone length. Growth in muscle and bony tissues is also subject to hormonal control. Pituitary growth hormone (somatotropin, SH) is most directly implicated in regulating such growth (de Bodo and Altszuler, 1957; Russell and Wilhelmi, 1958). However, for the optimal effects of growth hormone to be manifest insulin (Salter and Best, 1953; Munro, 1956; Russell and Wilhelmi, 1958), thyroid hormones (Simpson, et al., 1950; Russell and Wilhelmi, 1958), and androgenic steroids (Dorfman and Shipley, 1956; Russell and Wilhelmi, 1958) must be in proper balance.

Explorations into the influence of environmental conditions on metabolic processes have only recently begun. Evidence is accumulating which indicates that exposure to cold, for instance, results in adaptive changes in hormonal secretions which lead to alterations in metabolism of a positive survival value (Potter, 1958). It is highly probable that environmental conditions induce similar adaptive changes in hormonal secretions regulating growth.

The American chameleon, Anolis carolinensis, is an ideal animal in which to study environmental and hormonal factors regulating growth. The abundance of the species suggests that it is remarkably adapted to its environment. Thousands are shipped from southeastern Louisiana annually to pet shops, carnivals, research and instructional laboratories apparently without greatly affecting the population size (Gordon, 1956; Fox and Dessauer, 1958a). The ability

---

1Anolis is a primitive genus of the family IGUANIDAE. Mittleman (1942) classifies Anolis as an early branch off of the main line of iguanid evolution. Anolis is the largest genus of the family numbering over three hundred species of which only two are found in the United States.
of Anolis to change color, its most notable external characteristic, appears to be adaptive in nature and is correlated with the ambient temperature (Parker and Starratt, 1904), background color (Greenberg and Noble, 1944), and with time of the day (Rahn and Rosendale, 1941). These color changes are controlled in part by the endocrine secretion of the pituitary, melanophorotropic hormone and the adrenal medullary secretion, epinephrine (Kleinholz, 1938a, 1938b).

Less obvious are the anatomical and physiological changes which occur in Anolis in different seasons. Both behavioral patterns of the animal (Greenberg and Noble, 1944) and the size, histology and composition of its tissues may be described in terms of three distinct periods. (Figure 1) During the Growth and Reproduction period of spring and summer primary and secondary sex organs are enlarged and functional (Evans and Clapp, 1940; Dessauer, 1955b; Fox, 1958), animals increase in body length and in muscle tissue (Gordon, 1956), and endogenous caloric stores in the liver and fat bodies are minimal (Dessauer, 1953, 1955a, 1955b). In the Storage period of autumn primary sex organs are atretic and non-functional, growth in body length and muscle tissue is minimal, and calories stored in liver and fat bodies are at a maximum. During the Hibernation period of winter primary sex organs become reorganized, growth in body length and in muscle tissue is minimal, and caloric stores in liver and fat bodies diminish.

These changes appear to be adaptations to seasonal variations in food supply and climate (Dessauer, 1953, 1955a, 1955b) and are at least in part under the control of the photoperiod (Dessauer, 1955a; Fox and Dessauer, 1957, 1958b). For example, during the increasing
Figure 1. Summary of seasonal cycles in *Anolis* in diagrammatic form. Data are taken from the literature.
hours or maximum hours of daylight of the Growth and Reproduction period, endogenous stores in fat and carbohydrate are minimal but the food supply of insects (McClure, 1938) is high and appetite of the anole attains a maximum. Caloric intake in excess of maintenance requirements is utilized for growth and reproduction. In the fall season of decreasing light hours its food supply remains high and its appetite, though decreasing, remains somewhat elevated. Caloric intake in excess of maintenance needs is stored as fat and carbohydrate. At the peak of the Storage period enough endogenous calories are available in the liver and fat bodies to maintain the animal for about one month at average summer temperatures (Dessauer, 1955b). The hibernating anole utilizes these stores during the winter season of short daylengths when insects are less available and its appetite is minimal even at high temperatures which occur on occasion in the New Orleans area in winter.

Some evidence, already available, indicates that environmental conditions induce changes in hormonal secretions in Anolis which in turn regulate metabolic processes associated with growth. The association of endocrine secretions with environmental conditions in controlling color changes has already been mentioned. The histological study by Poris (1941) of pituitaries at different seasons indicated that the pituitary undergoes cyclic changes in secretory activity. The secretory activity of the thyroid also undergoes cyclic seasonal variations which may be altered by the hours of light to which the animal is exposed (Fox and Dessauer, 1959). A detailed study of the seasonal secretory cycles of interstitial cells of the testis of Anolis has been
reported (Fox, 1958). Fox and Dessauer (1958b) have also studied the effects of hours of light exposure on the secretory activity of interstitial cells of Anolis in different seasons.

Anolis lends itself to physiological studies for a number of additional reasons. Unlike most reptiles they may be captured in the wild in large numbers at any season in the year. Sexual differentiation is comparatively easy as the two enlarged scutes just posterior to the cloacal opening, the gular pouch and, in the breeding season, the nuchal crest are all present in the male and absent in the female (Smith, 1946). Juvenile males, a size still in the logarithmic state of growth, range in snout-vent length from 52 to 60 mm. and from 2.5 to 4 grams in body weight. Adult males, a size in which growth is very slow, range from 60 to 73 mm. in snout-vent length and from 4 to 7 grams in body weight (Dessauer, 1955b; Gordon, 1956). Animals this size are easy to handle and large numbers can be utilized in long term experiments without occupying excessive space. Chemical analyses can be performed on whole animals or whole organs rather than tissue samples, thus eliminating errors due to variations in composition from one portion of tissue to another. Early difficulties experienced in work with small animals have been diminished with the development of microchemical techniques. Meal worms, larvae of the beetle, Tenebrio molitor, are nutritionally adequate in maintaining Anolis in the laboratory for at least two months (Dessauer, 1953, 1955a; Fox and Dessauer, 1957, 1958b). Meal worms can be easily cultured in the laboratory (Cotton and St. George, 1929).
Although much evidence is available on *Anolis* concerning environmental influences upon growth and related seasonal metabolic changes, direct data implicating endocrines in control of growth are scarce. Experiments reported herein were designed on the assumption that growth is in part under endocrine control. It is postulated that, in a well adapted animal, environmental stimuli are translated into hormonal secretions which in turn control metabolic processes.
INTRODUCTION

Seasonal states which occur in Anolis can be altered or mimicked through control of the photoperiod. Exposure to 18 hours of light per 24 hour period stimulates growth, appetite and reproduction, and exposure to 9 hours of light per 24 hour period depresses growth, appetite and reproduction (Fox and Dessauer, 1957, 1958b). Consideration of the well established role of the hypophysis in regulating growth, appetite and reproduction suggests that these physiological changes are mediated through the hypophysis (Hammond, 1954). Thus anoles appear to be functionally hypophysectomized when exposed to 9 hours of light per 24 hour period, either naturally, as in winter, or experimentally. Experiments were designed to determine whether growth could be promoted in such "functionally hypophysectomized" anoles with hormones considered to be growth promoting in mammals.

1Presented before The Federation of American Societies for Experimental Biology in Chicago. (DiMaggio, 1960)
METHODS

Immature male anoles (52 to 62 mm. snout-vent length) collected in the New Orleans area were used in the experiments. "Functional hypophysectomy" was sustained or initiated by exposure of the animals to 6 hours of light per 24 hour period. The light-hours were shortened since some growth was reported for anoles exposed to 9 hours of light per 24 hour period. Artificial light was supplied by daylight fluorescent lamps mounted one and one-half feet above each cage. Cage temperatures were maintained at 28±2°C.

Animals were injected daily with either 0.1 ml. of distilled water or 0.1 ml. of an aqueous solution of the hormone(s). All animals were fed meal worms, larvae of *Tenebrio molitor*. Liberal amounts of worms were weighed and placed in the cages and food consumption was determined periodically from the weight of meal worms eaten. Caloric intake was estimated using the conversion factor 2 cal./mg. meal worms as derived from data on composition (Dessauer, 1955a). Cages were sprinkled with water daily. Periodically during the course of the experiments, animals were fasted three days and then weighed and measured. The duration of each experiment was approximately two months and at the end of the experimental period fasted animals were weighed, measured, and sacrificed by decapitation. Livers and fat bodies were weighed immediately on a torsion balance. Livers and carcasses were extracted with Bloor's mixture (3 vol. ethanol, 1 vol. ethyl ether), and the fat free residue dried to constant weight in a vacuum desiccator over concentrated sulphuric acid. The extract was evaporated and the lipid weighed.
Winter Experiment: January 1st to March 1st. Animals were divided into four groups of approximately equal size distribution. These groups were injected intraperitoneally as follows: (a) bovine growth hormone,\(^2\) 10 mg./kg./day, (b) desiccated bovine thyroid powder, 7 mg./kg./day, (c) a mixture of growth hormone and thyroid powder at the same dosage as in (a) and (b), (d) distilled water, 0.10 ml./animal/day. Each group was kept in a separate cage.

Summer Experiment: May 1st to July 5th. Animals were divided into five groups of approximately equal size distribution. These groups were injected intraperitoneally as follows: (a) bovine growth hormone,\(^2\) 10 mg./kg./day, (b) desiccated bovine thyroid powder, 7 mg./kg./day, (c) bovine insulin,\(^3\) 25 mg./kg./day, (d) a mixture of bovine growth hormone, bovine thyroid powder and bovine insulin at the same dosages, (e) distilled water, 0.10 ml./animal/day.

As a complex social order exists in Anolis food intake of smaller animals is influenced by the presence of larger animals in the same cage. In an attempt to minimize such influences animals were placed in individual white wooden boxes, 7 cm. x 12 cm. x 12 cm.; with screen wire covers.

\(^2\)Somar, Armour. Gift of Endocrinology Study Section of the National Institutes of Health.

\(^3\)Iletin, Lilly.
RESULTS

Effect of Hormones on Body Weight. An increase in body weight occurred in both experiments (Table I). In the winter experiment the weight increase of animals given growth hormone, desiccated thyroid or a combination of both hormones was greater than that of the controls. The weight differences between experimentals and controls were statistically significant for groups given growth hormone (P = .05) or a combination of growth hormone and desiccated thyroid (P = .05) if percentage changes in weight were compared. However, if compared on the basis of absolute changes in body weight, the differences did not prove to be statistically significant (P > .05). In the summer experiment, with a larger sample size, statistically significant differences from the controls were obtained on both the absolute and percentage basis with growth hormone (absolute: P = .05; relative: P < .01), thyroid powder (absolute: P = .05; relative: P = .05), and a combination of growth hormone, desiccated thyroid and insulin (absolute: P = .05; relative: P = .05). The weight gain of both control groups was of the same order as that reported on animals exposed to 9 hours of light per 24 hours, but with less variation (Fox and Dessauer, 1957).

Mean growth curves for groups of the summer experiment are presented in Figure 2. The curves for the thyroid and insulin groups, not shown in Figure 2, were similar to those for growth hormone and control groups respectively. The total increase in weight of the control group occurred during the first week, whereas experimental groups continued to increase in weight for at least a month. The rate of weight increase of animals given a combination of growth hormone, thyroid powder and insulin approximated that reported for anoles.
<table>
<thead>
<tr>
<th>Series</th>
<th>Sample Size</th>
<th>Snout-Vent Length</th>
<th>Body Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Original Growth</td>
<td>% Sample Growth 2 mm. or More</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Millimeters</td>
<td>Winter</td>
</tr>
<tr>
<td>Controls</td>
<td>6</td>
<td>53.0±3.3(^1)</td>
<td>0.7±1.3(^2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50-56(^2)</td>
<td>-1.2</td>
</tr>
<tr>
<td>Growth</td>
<td>5</td>
<td>55.6±2.5</td>
<td>1.8±1.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>52-59</td>
<td>0-4</td>
</tr>
<tr>
<td>Thyroid</td>
<td>5</td>
<td>55.3±3.7</td>
<td>2.6±2.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50-58</td>
<td>0-6</td>
</tr>
<tr>
<td>Growth and Thyroid</td>
<td>5</td>
<td>55.6±3.2</td>
<td>2.1±1.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>52-60</td>
<td>0-4</td>
</tr>
<tr>
<td>Controls</td>
<td>14</td>
<td>57.1±2.4</td>
<td>0.9±1.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>52-61</td>
<td>0-3</td>
</tr>
<tr>
<td>Growth</td>
<td>14</td>
<td>57.1±3.2</td>
<td>3.1±1.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>51-61</td>
<td>0-5</td>
</tr>
<tr>
<td>Thyroid</td>
<td>14</td>
<td>57.9±3.3</td>
<td>1.1±1.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>52-61</td>
<td>0-6</td>
</tr>
<tr>
<td>Insulin</td>
<td>11</td>
<td>59.0±2.2</td>
<td>2.0±1.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>52-62</td>
<td>0-6</td>
</tr>
<tr>
<td>Growth, Thyroid and Insulin</td>
<td>9</td>
<td>59.5±2.0</td>
<td>3.1±1.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>57-63</td>
<td>1-5</td>
</tr>
</tbody>
</table>

\(^1\)Mean ± Standard Deviation  \(^2\)Range
Figure 2. Mean growth of control and hormone injected *Anolis* conditioned to a photoperiod of only 6 hours of light per day.
exposed to 18 hours of light per 24 hour period (Fox and Dessauer, 1957).

In an attempt to distinguish fat deposition from true growth, composition of the weight added during the summer experimental period was estimated as follows (Table II): Non-lipid weight gain was calculated by subtracting combined liver and fat body lipid from total weight gain. This is a reasonable estimation because the quantity of lipid in the carcass, exclusive of that in the liver and fat bodies, is relatively constant throughout the year. The marked seasonal changes in lipid content occur primarily in the liver and fat bodies (Dessauer, 1955b). Lipid is absent from livers and fat bodies in spring (Dessauer, 1955b). 4 As the summer experiments began in early May, lipid deposited during this experimental period equaled that present in the liver and fat bodies at the time of autopsy.

Non-lipid dry weights were 23-25% of non-lipid wet weights for all groups. Thus non-lipid weight gain of experimental groups was presumably due to protein of approximately the same hydration as proteinaceous tissues such as the liver (Dessauer, 1955b) and not due to abnormal water retention. Protein weight gain was estimated as 23-25% of non-lipid weight gain with an error probably no greater than 5%.

As weight increase due to lipid was the same magnitude for all groups, the greater increase in weight of hormone injected anoles was due largely to increased protein retention.

---

4 The absence of lipid from livers and fat bodies of 24 anoles captured in early May confirmed the observation of Dessauer (1955b).
Table II
Caloric Efficiency and Estimation of True Growth

<table>
<thead>
<tr>
<th>Summer Group</th>
<th>Total</th>
<th>Lipid</th>
<th>Non-Lipid</th>
<th>Protein</th>
<th>Weight Gain in mg.</th>
<th>Weight Gain in mg./g. Meal Worms Eaten</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>250</td>
<td>113</td>
<td>137</td>
<td>33</td>
<td></td>
<td>96±72</td>
</tr>
<tr>
<td>Growth Hormone</td>
<td>650</td>
<td>94</td>
<td>556</td>
<td>129</td>
<td></td>
<td>216±140</td>
</tr>
<tr>
<td>Thyroid</td>
<td>460</td>
<td>110</td>
<td>350</td>
<td>87</td>
<td></td>
<td>216±61</td>
</tr>
<tr>
<td>Insulin</td>
<td>170</td>
<td>100</td>
<td>70</td>
<td>17</td>
<td></td>
<td>70±104</td>
</tr>
<tr>
<td>Growth, Thyroid and Insulin</td>
<td>930</td>
<td>117</td>
<td>813</td>
<td>190</td>
<td></td>
<td>219±163</td>
</tr>
</tbody>
</table>
Effect of Hormones on Snout-Vent Length. Increase in snout-vent length occurred in all groups (Table I). However, the mean increase of the controls was within the experimental error of the measurement. In comparison with the controls statistically significant changes in snout-vent length occurred only in groups of the summer experiment that received growth hormone, \( P < .01 \) or a combination of growth hormone, desiccated thyroid and insulin \( P < .01 \). The absence of any statistically significant differences in groups of the winter experiment could be attributed to small sample size and high variation.

Effect on Energy Metabolism. Although animals were fed ad libitum, no differences in total food consumption between the control and experimental groups were detected in either the summer or winter experiments. The calculated mean caloric intake for each summer group was 40 cal./g. original body weight/day. Food consumption of individual animals in the winter experiment could not be measured since each cage housed eight animals originally and two or three animals from each group died at various intervals. However, assuming that the animals that died were not eating, the caloric intake for each group was also nearly identical, averaging 41-42 cal./g. original body weight/day.

With the exception of the summer insulin groups, hormone injected animals gained more weight per gram of meal worms ingested than control animals. These data for the summer experiment are presented in Table II. Differences in weight gained per gram of meal worms eaten between the control group and groups injected with growth hormone \( P = 0.01 \), desiccated thyroid \( P = .05 \), and a combination of growth hormone, desiccated thyroid, and insulin \( P = .01 \) were statistically significant.
INTRODUCTION

A paradox appears to exist when one considers seasonal differences in growth rates and energy stores of Anolis. Growth is maximum in spring and summer when fat stores of the anole are so low that if starved it must utilize body protein to meet standard metabolic requirements. To quantitate this contribution of endogenous protein oxidation in fulfilling standard metabolic requirements, nitrogen excretion was measured in fasted anoles at different seasons. Rate of disposal of a nitrogen load imposed by feeding was also measured throughout the year.

Preliminary findings on nitrogen excretion showed that protein catabolism was low in late autumn and winter, seasons during which tissue carbohydrate stores were high. Less endogenous protein is oxidized by many animals if carbohydrate is high in their diets (protein sparing effect of carbohydrates). Does the elevation of tissue carbohydrate of Anolis during seasons of low protein intake represent a metabolic adaptation leading to conservation of tissue protein? To test this hypothesis nitrogen excretion rates of anoles fed glucose were compared to rates in fasted controls.
As growth hormone stimulated growth in "functionally hypophysectomized" animals (Chapter II), experiments were designed to further assay the effects of growth hormone on nitrogen metabolism. Anoles were injected with growth hormone and their nitrogen excretion rates were measured while fasting and after being fed a single high energy, high protein meal.

METHODS

Collection and Determination of Nitrogen. Although the major nitrogen excretory product in Anolis is uric acid, about 10% of the total nitrogen is in the form of ammonium ion (Dessauer, 1952). To prevent loss of ammonia nitrogen during collection, special containers were prepared. One ml. of a concentrated solution of citric acid was layered over the bottom of a 400 ml. beaker. The beaker was heated until the solution began to boil. Upon cooling a fine layer of citric acid crystals was deposited uniformly over the bottom of the beaker. Each animal was placed in a beaker directly on the citric acid. Any urinary ammonia excreted was converted immediately to non-volatile ammonium citrate. The animals, in wire mesh covered coated beakers, were kept in a dark incubator at \(28^{\pm}0.5^\circ\text{C.}\) during the collection periods. Empty citric acid coated beakers placed in the incubator during the collection periods served as blanks. Placing the animals directly on the solid citric acid caused no ill effects unless the humidity increased to such a value as to cause solution of the citric acid crystals. This problem was overcome by maintaining.
the relative humidity at 60% by placing a pan of potassium dichromate crystals in the incubator.

At the beginning and end of each collection period, urine and feces were washed from the cloaca and the final washings, together with excreta already in the beakers, were transferred to a 50 ml. digestion tube. Kjeldahl digestions were performed on the blanks and on excreta from each animal. Nitrogen was determined on aliquots of the digests by the microdiffusion method of Conway (1940). Blanks gave values on the order of 5% of the total urinary nitrogen.

Collection of Animals. Population samples of immature male anoles were collected in the New Orleans area each month for a period of one year. For four days the animals were maintained in the laboratory at a constant temperature of 28±2°C. and exposed to the normal photoperiod for the time of their capture at latitude 30° north. The animals were fasted but their cages were sprinkled daily with water.

Fasting Nitrogen Excretion Rates. On the fourth day after capture animals were weighed, measured and excreta washed out of the cloaca with distilled water. Collection of excreta was begun at this time for the determination of fasting base-line nitrogen excretion rates. During the collection period animals were not fed but were injected daily with 0.1 ml. of distilled water to maintain them in a good state of hydration.

Nitrogen Excretion After Feeding Meal Worms. After fasting nitrogen excretion rates were determined, the same anoles were force fed 72 mg. meal worms/g. body weight. One tenth milliliter of water was injected intraperitoneally daily into each animal during the collection period. Excreta was collected and nitrogen determined. The amount of meal worms
fed provided enough calories to meet the standard metabolic requirements for six days as calculated from meal worm composition (Dessauer, 1955a) and a standard metabolic rate of 24 cal./g./day based on O2 utilization (Dessauer, 1953). Periodically samples of meal worms were analyzed for nitrogen using the same method as for urinary nitrogen. They were found to contain $2.22\pm0.05$ micromole N/mg. meal worm or the equivalent of 20% protein. This value is in good agreement with literature values for meal worm composition (Mellanby, 1932; Dessauer, 1955a).

**Nitrogen Excretion After Feeding Glucose.** The effect of glucose on nitrogen excretion was measured in February, July and August. At the beginning of the three day collection period anoles were fed by stomach tube 25 mg. glucose in 0.2 ml. aqueous solution. Excreta was collected and nitrogen determined as described above.

**Effect of Growth Hormone on Nitrogen Excretion.** Periodically throughout the year the effect of bovine growth hormone on nitrogen excretion in fasting and fed anoles was determined. The procedures were the same as described for fasting and feeding experiments except that animals were divided into two groups. Controls were injected daily with water; experimentals were injected on each day of the collection period with 0.1 ml. growth hormone solution at a dosage of 10 mg./kg./day.
RESULTS

When measurements on fasting nitrogen excretion were begun collections of excreta were made every 24 hours. It was found that the excretion of any particular animal was relatively constant with a variation of about 5% for several consecutive days. To minimize experimental errors mean nitrogen excretion rates of each animal were calculated from analysis of a single sample collected over a period of three days. The mean nitrogen excretion rate, its standard deviation, and range for each month are presented in Table III and Figure 3.

Nitrogen loss in fasting anoles was at a minimum in autumn, increased steadily to maximum in spring, and decreased to a plateau in summer. Individual variations from monthly means were of the order of 25% to 35%. Greatest individual variations occurred in October (43%) when the sample size was largest, and in February 1959 (56%). Individual variations in February 1960, with a larger sample size, were reduced to 27%. Although individual variations were high, differences in nitrogen excretion rates between autumn and spring, spring and summer, and summer and autumn anoles were all highly significant (P < .01). Injections of 10 mg./kg./day growth hormone for three days did not alter nitrogen excretion rates of fasting animals in any season (Table III).

In December 1958 four large anoles were fed enough meal worms to provide calories for six days at the standard metabolic rate. Nitrogen excretion was greatly elevated on the first and second day after feeding, slightly elevated on the third, and was the original level four to eight days later. Only 72% of the nitrogen contained in
### TABLE III

**NITROGEN EXCRETION RATES OF ANOLIS**

<table>
<thead>
<tr>
<th>Date</th>
<th>Fasting Hormone</th>
<th>Glucose</th>
<th>Feeding</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Micrograms N/gram Body Weight/Day</td>
<td>% Dietary N/3 Days</td>
<td></td>
</tr>
<tr>
<td>1 Feb 59</td>
<td>(6)12.3±4.92</td>
<td>5.3-25.83</td>
<td>(6)77±14</td>
</tr>
<tr>
<td>1 Feb 60</td>
<td>(17)13.2±3.5</td>
<td>6.6-19.5</td>
<td>63-86</td>
</tr>
<tr>
<td>5 Feb 60</td>
<td>(7)14.0±3.8</td>
<td>(11)5.3±1.1</td>
<td>3.2-7.1</td>
</tr>
<tr>
<td>1 Mar 59</td>
<td>(8)16.1±4.3</td>
<td>8.5-22.8</td>
<td>8.5-22.8</td>
</tr>
<tr>
<td>1 Apr 59</td>
<td>(7)21.0±3.2</td>
<td>8.9-22.4</td>
<td>8.5-22.8</td>
</tr>
<tr>
<td>15 May 59</td>
<td>(16)24.8±7.7</td>
<td>11.3-42.5</td>
<td>(16)123±27</td>
</tr>
<tr>
<td>10 Jun 59</td>
<td>(16)14.9±5.8</td>
<td>(3)14.7±4.3</td>
<td>(8)83±21</td>
</tr>
<tr>
<td>15 Jul 59</td>
<td>(20)16.2±5.5</td>
<td>6.5-29.6</td>
<td>62-124</td>
</tr>
<tr>
<td>21 Jul 59</td>
<td>(10)13.1±4.3</td>
<td>8.4-21.4</td>
<td>7.8-20.3</td>
</tr>
<tr>
<td>7 Aug 59</td>
<td>(18)16.0±4.9</td>
<td>8.5-29.6</td>
<td>7.7-22.1</td>
</tr>
<tr>
<td>7 Aug 60</td>
<td>(6)17.7±3.8</td>
<td>11.1-27.4</td>
<td>(19)9.3±2.8</td>
</tr>
<tr>
<td>10 Sep 59</td>
<td>(7)10.0±3.0</td>
<td>7.1-15.1</td>
<td>5.1-15.0</td>
</tr>
<tr>
<td>15 Oct 60</td>
<td>(48)8.2±3.5</td>
<td>4.9-15.0</td>
<td>56-67</td>
</tr>
<tr>
<td>19 Oct 60</td>
<td>(12)5.8±1.4</td>
<td>4.0-7.2</td>
<td>5.7-9.3</td>
</tr>
<tr>
<td>5 Nov 60</td>
<td>(38)9.3±3.3</td>
<td>4.2-16.0</td>
<td>5.7-13.1</td>
</tr>
<tr>
<td>8 Nov 60</td>
<td>(5)8.1±3.0</td>
<td>5.7-13.1</td>
<td>(8)76±7</td>
</tr>
<tr>
<td>15 Dec 59</td>
<td>(15)10.0±3.2</td>
<td>5.5-14.7</td>
<td>67-86</td>
</tr>
<tr>
<td>18 Dec 59</td>
<td>(8)8.6±2.0</td>
<td>(7)9.4±3.7</td>
<td>5.5-14.5</td>
</tr>
</tbody>
</table>

1 Number of Animals  
2 Mean ± Standard Deviation  
3 Range  
4 Three to six days after glucose
Figure 3. Mean nitrogen excretion rates of Anolis at different seasons. Circles connected by a continuous line represent excretion during a fast. Horizontal bars at the ends of the arrows represent excretion of animals fed glucose.
the food was excreted during the three days when the excretion rate was elevated. This pattern of elevated nitrogen excretion for only three days after feeding was repeatedly obtained after feeding various amounts of protein and in all seasons. The percentage of a constant dietary nitrogen load excreted in three days was used as a basis for seasonal comparison of the efficiency of nitrogen disposal.

The ability of anoles to excrete a nitrogen load imposed by feeding meal worms parallels fasting nitrogen excretion rates (Figure 4 and Table III). In autumn when fasting nitrogen excretion rates were low, only 61% of ingested nitrogen was excreted in three days. In spring, when fasting rates were elevated, nitrogen excretion in fed animals exceeded nitrogen fed. Differences in rates of excretion of dietary nitrogen were statistically significant between autumn and winter, winter and spring, spring and summer and summer and autumn (P = .01). Individual variations from means ranged from 7-8% in autumn when excretion was low to 20-25% in spring when excretion was maximum.

Anoles fed meal worms and injected intraperitoneally with growth hormone on each day during the collection period excreted higher percentage of dietary nitrogen in three days than uninjected control anoles (Table IV). Differences in excretion rates between uninjected control anoles and growth hormone injected anoles proved to be statistically significant, except in December (Table IV).

Nitrogen excretion was sharply decreased in anoles fed glucose (Table III). In July and August glucose feeding decreased the nitrogen excretion rate from 16 µg. N/g. body weight/hour to 10 µg. N/g. body weight/hour, the nitrogen excretion rate of fasting anoles in September.
Figure 4. Percentage of a constant amount of dietary nitrogen fed in a single meal excreted in three days. Horizontal I equals the mean, vertical I represents the range, solid vertical bar indicates the Standard Deviation.
<table>
<thead>
<tr>
<th>Date</th>
<th>Group</th>
<th>Number of Animals</th>
<th>Nitrogen Load mg. N/gram Body Weight</th>
<th>Percent Dietary Nitrogen Excreted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feb. 1960</td>
<td>Control</td>
<td>7</td>
<td>1.47</td>
<td>83±8</td>
</tr>
<tr>
<td></td>
<td>Growth</td>
<td>9</td>
<td>1.47</td>
<td>111±17 (P = .01)</td>
</tr>
<tr>
<td>June 1959</td>
<td>Control</td>
<td>8</td>
<td>1.85</td>
<td>83±21</td>
</tr>
<tr>
<td></td>
<td>Growth</td>
<td>7</td>
<td>1.85</td>
<td>111±14 (P = .05)</td>
</tr>
<tr>
<td>Oct. 1959</td>
<td>Control</td>
<td>6</td>
<td>2.22</td>
<td>61±4</td>
</tr>
<tr>
<td></td>
<td>Growth</td>
<td>7</td>
<td>2.22</td>
<td>72±6 (P = .01)</td>
</tr>
<tr>
<td>Nov. 1959</td>
<td>Control</td>
<td>5</td>
<td>1.47</td>
<td>64±5</td>
</tr>
<tr>
<td></td>
<td>Growth</td>
<td>5</td>
<td>1.47</td>
<td>80±16 (P = .05)</td>
</tr>
<tr>
<td>Dec. 1959</td>
<td>Control</td>
<td>8</td>
<td>2.22</td>
<td>76±7</td>
</tr>
<tr>
<td></td>
<td>Growth</td>
<td>7</td>
<td>2.22</td>
<td>69±10 (P &gt; .30)</td>
</tr>
</tbody>
</table>
In February glucose feeding decreased the nitrogen excretion rate from 13\,\mu g. N/g. body weight/hour to 5\,\mu g. N/g. body weight/hour, a rate lower than the minimum obtained in October. Individual variations from the mean were considerably lower in glucose fed animals than in the control animals in February and July, but not in August. Differences in nitrogen excretion rates between fasting and glucose fed anoles were highly significant (P < .01).
GLUCOSE METABOLISM STUDIES

INTRODUCTION

Growth processes cannot be fully understood without knowledge of the control of metabolic processes in general or of carbohydrate metabolism in particular. For example, a major intermediate of carbohydrate metabolism, glucose, has protein sparing action, can contribute portions of its molecule to the structure of protein as well as to the structure of glycogen and fat and is, usually, readily utilized as an energy source by living tissues.

Blood glucose of an animal does not of itself give a dynamic picture of carbohydrate metabolism as the value is influenced by many variables. These include rate of glycogen and fat synthesis, rate of carbohydrate oxidation, rate of glycogenolysis, etc. On the other hand, experiments designed to follow the disposition of a constant amount of injected glucose by an animal should give evidence on: (1) rate of glycogen synthesis, (2) rate of glycogen breakdown, (3) rate of change with time of blood sugar (glucose tolerance). Such experiments have been carried out at different times of the year in an attempt to obtain further evidence of underlying factors responsible for observed seasonal differences in gross tissue composition of Anolis.
Pancreas, pituitary, thyroid and adrenal glands are known to affect carbohydrate metabolism as well as growth in mammals and other vertebrates. Carbohydrate distribution was determined after injections of: (1) a pituitary secretion, growth hormone; (2) the pancreatic secretions, insulin and/or glucagon; and, (3) the adrenal secretions, hydrocortisone and epinephrine.

METHODS

Population samples of from thirty to fifty large, adult male American chameleons, Anolis carolinensis, 3.5 to 7 grams body weight were collected in the New Orleans area at different times of the year. Lizards were maintained in the laboratory for four days at a constant temperature of 28±2°C. and exposed to the normal photoperiod for the time of the year of their capture at latitude 30° north. The animals were fasted but their cages were sprinkled daily with water.

Seasonal Glucose Disposition. On the fourth day after capture lizards were divided into groups of from five to six individuals, matched on the basis of body weight and snout-vent length. One group was sacrificed at this time for base line organ weights and chemical analyses. A second group, in most population samples, was injected intraperitoneally with 0.4 ml. of water. Three to four other groups were injected intraperitoneally with 40 mg. of glucose in 0.4 ml. of solution. These animals, in 400 ml. beakers covered with wire mesh, were placed in a dark incubator, 28±0.5°C., and remained undisturbed until time of sacrifice. They were without either food or water during this period.
At various intervals of time groups of glucose injected lizards were sacrificed and their tissues analyzed for carbohydrate. The untreated controls, the water injected lizards, were sacrificed along with the last glucose injected group. At time of sacrifice any urine or feces present in the cloaca was washed out with distilled water. This collection was pooled with other excreta of the lizard and analyzed to estimate glucose loss in urine. No special precautions were taken to prevent bacterial action, however, urine was scant and dried rapidly. Animals were decapitated and 0.05 to 0.10 ml. of blood allowed to flow directly into a micro blood pipette. Measured samples were added to tungstate deproteinizing solution. The animal was cut open ventrally and any peritoneal fluid present was collected and also deproteinized in tungstate solution. The liver was removed quickly, weighed on a torsion balance and dropped into 1 ml. of boiling 20% KOH. Fat bodies and testes were weighed and, with the head and remainder of the carcass, were minced and dropped into 3 ml. of boiling 20% KOH.

Glucose in the protein-free filtrates of blood, urine, and peritoneal fluid was determined by a micro modification of the method of Folin and Wu (1920). Livers and carcasses were digested in the hot 20% KOH for two hours. Glycogen was precipitated by adding 1.2 volumes of 95% ethanol to the hot digests and allowing the tubes to stand in the refrigerator for at least twelve hours. After centrifugation and decantation the precipitate was washed with 95% ethanol, recentrifuged and washings allowed to drain off. The glycogen was dissolved in a known volume of warm water. Insoluble melanoid pigments and bones were removed from suspension in the glycogen solution by centrifugation. Glycogen in the supernatant was determined by means of the pheno-sulfuric acid method of Montgomery (1957).
Determination of Hormonal Effects on Blood Glucose and Tissue Glycogen. Four days after capture animals were divided into groups as previously described. Animals of these groups were injected intraperitoneally with either 0.2 ml. of distilled water or 0.2 ml. of an aqueous solution of the hormone(s) daily for three or four days. Hormones injected were: (1) bovine insulin (Iletin, Lilly), (2) bovine glucagon-free insulin (GF insulin), (3) bovine glucagon, (4) a combination of glucagon and GF insulin, (5) epinephrine, (6) bovine thyroid powder, (7) bovine growth hormone, (8) a combination of growth hormone and hydrocortisone. In other experiments animals of each group were injected with .04 ml. of a 10% glucose solution on the fourth day after capture. Animals of the experimental groups were injected with insulin or glucagon 48 and 72 hours after being injected with glucose; control animals were injected with distilled water. Hormone injected animals were also kept in a dark incubator at 28±0.5°C. Animals were sacrificed 24 hours after the last hormone injection. Sacrifice procedures and analytical methods were the same as for experiments on seasonal glucose disposition. Qualitative urine analyses for glucose and ketone bodies using Testape and Acetest were performed on animals injected with a combination of growth hormone and hydrocortisone.

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1Iletin is known to contain glucagon activity.

2Crystalline glucagon (Lot #258-234B-54-2) and glucagon-free insulin were kindly supplied by the Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana.
Tissues of animals in the hormonal replacement studies (Chapter II) were also analyzed for carbohydrate content. Blood was collected and analyzed for glucose by the same methods used in the seasonal glucose disposition experiments. Livers and carcasses were immediately dropped in hot Bloor's solution. After fat extraction and desiccation to constant weight, livers and carcasses were digested in hot 20% KOH for two hours. Glycogen in the digests was determined as above.

RESULTS

GLUCOSE DISPOSITION EXPERIMENTS:

**Glucose Tolerance.** The rate of decrease of reducing substance (glucose) in the blood varied markedly at different seasons of the year. In animals studied during the months of March through July less than 36 hours were required for blood glucose to return to the fasting level after a 40 mg. load of glucose (Figure 5). During autumn and winter, however, blood glucose remained considerably elevated above fasting level for over two days. As there is evidence for seasonal variations in fasting blood glucose in the species (Dessauer, 1952), we have plotted differences in glucose between fasted controls and glucose-injected animals. Based upon the average level of glucose difference at 24 and 48 hours (Figure 6), it is apparent that this lizard can clear glucose from its blood most rapidly during spring and summer. During August and September, however, a distinct decrease in "tolerance" for
Figure 5. Variations in glucose tolerance with season.
Figure 6. Differences in blood glucose level between control and glucose injected animals (A) 24 hours after glucose injection, (B) 48 hours after glucose injection. Horizontal I equals the mean, vertical I represents the range, vertical solid bar indicates the Standard Deviation.
glucose becomes apparent. The tolerance progressively decreases until it attains a minimum in the December animals.

**Liver Glycogen.** Marked changes in the amount of glycogen deposited in the liver and in the rate of glycogen breakdown accompanied seasonal alterations in glucose tolerance (Figure 7). After a 40 mg. injection of glucose in spring and summer, liver glycogen increased to a maximum of about 5% liver weight in 24 hours. It then decreased rapidly and approached the low fasting level of 0.2% to 0.6% liver weight after 72 hours. During autumn and to a less extent in winter, glycogen concentration reached a maximum of from 5% to 10% liver weight in 48 hours. Most striking was the change in the rate at which glycogen disappeared from these livers. In contrast to livers of animals in spring and summer, livers in fall and winter tended to retain glycogen for long periods. This was especially marked in livers of anoles in October and November in which, four days after glucose injection, livers still averaged more than 5% glycogen. Since such livers are two to three times larger at this season than those in the spring, the absolute amount of glycogen retained was even more striking when compared to animals in spring and summer. Even non-injected controls in captivity eight days without food had 2% to 3% glycogen in their livers which still averaged 3% to 5% body weight (Figure 8). Livers of similarly starved animals in July were only 2.2% body weight and contained an average of only 0.36% glycogen.

**Carcass Glycogen.** Marked changes in the amount of glycogen deposited in non-hepatic tissues and in the rate of its breakdown accompanied seasonal alterations in glucose tolerance (Figure 9). Seasonal variations in base-line carcass glycogen are presented in Figure 10. After a 40 mg.
Figure 7. Seasonal variations in the rates of glycogen deposition in and utilization by the liver. Top line connects means for animals studied in October; bottom line connects means for animals studied in July. Vertical I represents the range, vertical solid bar indicates the Standard Deviation.
Figure 8. Average content of glycogen in livers of Anolis in different seasons: (a) 24 hours after glucose injections, (b) 72 hours after glucose injections, (c) after a 7 to 8 day fast.
Figure 9. Seasonal variations in the rate of glycogen deposition in and utilization by non-hepatic tissues.
injection of glucose in July, carcass glycogen increased to a maximum of 2 mg./g. body weight in 24 hours, then decreased rapidly and approached a low fasting level of 1 mg./g. body weight by 72 hours. Carcass glycogen was at a maximum of 5 mg./g. body weight in October (Figure 10). Injection of glucose did not significantly raise this value. In winter baseline carcass glycogen was reduced to 2 mg./g. body weight. After glucose injection carcass glycogen increased in 24 hours to the same level as in October and decreased slightly in 72 hours. Carcass glycogen, like liver glycogen, was extremely stable in autumn but disappeared more rapidly in summer. In October carcass glycogen in non-injected controls fasted for eight days was slightly greater than in controls fasted for four days. In July, however, carcasses of anoles fasted eight days contained only half as much glycogen as carcasses of anoles fasted four days.

Urinary Glucose. Marked seasonal differences in the amount of glucose excreted in the urine were also obtained (Figure 11). Of the 40 mg. of glucose injected an average of only 1 mg. was lost in excreta of December animals in contrast to an average of 15 mg. lost in excreta of animals injected in May.

EFFECTS OF GLUCAGON AND INSULIN ON TISSUE CARBOHYDRATES (Table V):

Blood Glucose. Glucagon in doses of 1, 5 and 10 mg./kg. body weight stimulated a marked rise of blood glucose. The response of fasting anoles to a constant dose of 5 mg./kg. glucagon increased from an elevation of 70 mg. % in August to an elevation of 270 mg. % in November. A maximum elevation of 540 mg. % was obtained in September with 10 mg./kg. glucagon injected into anoles which had been injected with glucose four days earlier. Although no significant changes in blood glucose was obtained with 25 mg./kg. Iletin, the same dosage of glucagon-
Figure 10. Glycogen in non-hepatic tissues of four day fasted Anolis in different seasons determined by the method of Montgomery (1957). Horizontal I equals the mean, vertical I represents the range, vertical solid bar indicates the Standard Deviation. Dots represent non-hepatic glycogen determined by the method of Good, et. al. (1933).
Figure 11. The manner of disposition of glucose by Anolis in different seasons. The chart attempts to depict the distribution of 40 mg. of glucose 48 hours after injection.
free insulin resulted in a small but statistically significant reduction in blood glucose.

In an attempt to obtain some measure of the relative effectiveness of glucagon and insulin, animals were simultaneously injected with 25 mg./kg. insulin and glucagon at various dosages. One mg./kg. body weight of glucagon abolished the effect of insulin and produced a net elevation in blood glucose.

**Liver and Carcass Glycogen.** Glucagon injections resulted in a decrease in liver glycogen. The reduction in liver glycogen proved to be statistically significant except in August and September. Data on the effect of glucagon on carcass glycogen were inconclusive.

Injections of glucagon-free insulin resulted in a significant decrease in liver glycogen (P = .05), and a significant increase in carcass glycogen (P < .01). Glucagon and insulin in combination appeared to act synergistically in reducing liver glycogen and increasing carcass glycogen. However, the differences between controls and hormone injected animals did not prove to be statistically significant.

**EFFECT OF OTHER HORMONES ON TISSUE CARBOHYDRATES (Table VI):**

**Blood Glucose.** Injections of epinephrine and of desiccated thyroid stimulated a rise in blood glucose of 20 and 30 mg. %. The differences in blood glucose levels between control and hormone injected animals did not prove to be statistically significant. Although growth hormone alone did not affect blood glucose, a combination of growth hormone and hydrocortisone stimulated a rise in blood glucose of 76 mg. % (P = .05). Neither glucose nor ketone bodies were detected in the urine of animals injected with a combination of growth hormone and hydrocortisone.
<table>
<thead>
<tr>
<th>Date</th>
<th>No.</th>
<th>Group</th>
<th>Dosage mg./kg.</th>
<th>Body Wt.</th>
<th>Blood Glucose mg. %</th>
<th>Glycogen mg./g. Body Weight</th>
<th>Liver</th>
<th>Carcass</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting Anoles</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 Aug 60</td>
<td>6</td>
<td>Control</td>
<td>215±34</td>
<td>0.22±.09</td>
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<td>6</td>
<td>Glucagon</td>
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<td>0.19±.12</td>
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<td>23 Sep 60</td>
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<td>Control</td>
<td>215±18</td>
<td>0.27±.13</td>
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<td>542±254</td>
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<td>12 Oct 59</td>
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<td>Control</td>
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<td>6</td>
<td>Iletin</td>
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<td>0.67±.34</td>
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<tr>
<td>10 Nov 59</td>
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<td>Control</td>
<td>134±09</td>
<td>0.34±.08</td>
<td>1.47±.12</td>
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<td>Glucagon</td>
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<td>0.38±.11</td>
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<td>5</td>
<td>Glucagon</td>
<td>403±150</td>
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<td>1.62±.19</td>
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<td>5</td>
<td>GF Insulin</td>
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<td>5</td>
<td>GF Insulin</td>
<td>217±69</td>
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<td>1.81±.29</td>
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<td></td>
<td>5</td>
<td>GF Insulin</td>
<td>305±64</td>
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<td>GLucose Injected Anoles</td>
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<tr>
<td>8 Aug 60</td>
<td>6</td>
<td>Control</td>
<td>216±13</td>
<td>0.40±.26</td>
<td>6.8±1.7</td>
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<td>Glucagon</td>
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<tr>
<td>23 Sep 60</td>
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<td>Control</td>
<td>240±34</td>
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<td>Glucagon</td>
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<td>21 Jan 60</td>
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<td>Control</td>
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<tr>
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<td>Glucagon</td>
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1Mean ± Standard Deviation
<table>
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<tr>
<th>Date</th>
<th>No.</th>
<th>Group</th>
<th>Dosage mg./kg.</th>
<th>Blood Glucose mg.%</th>
<th>Glycogen mg./g. Body Weight</th>
<th>Body Wt.</th>
<th>Liver</th>
<th>Carcass</th>
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<tr>
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<td>Epinephrine</td>
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<td>Control</td>
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<td>9</td>
<td>Growth</td>
<td>10</td>
<td>163±25</td>
<td>0.24±0.16</td>
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<td></td>
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<tr>
<td></td>
<td></td>
<td>+ Insulin</td>
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1 Mean ± Standard Deviation
2 Hormonal Replacement Experiment
Liver and Carcass Glycogen. Injections of epinephrine resulted in a decrease in both liver and carcass glycogen. However, the reduction in tissue glycogen was not statistically significant, possibly due to the greater individual variations observed in the control animals. Coefficients of variation for control animals were twice the value obtained for experimental animals. Injections of desiccated thyroid produced no measurable effects on either liver or carcass glycogen. Liver glycogen was decreased following injections of growth hormone in January \( (P = .05) \) but not in February (no difference). Glycogen content of livers of anoles injected with a combination of growth hormone and hydrocortisone was only 10% less than that of livers of control animals, well within the experimental error of the measurements. Carcass glycogen was significantly reduced in anoles injected with growth hormone alone and in anoles injected with a combination of growth hormone and hydrocortisone \( (P = .01) \).

**TISSUE CARBOHYDRATES IN HORMONAL REPLACEMENT EXPERIMENTS** (Table VI):

**Blood Glucose.** Daily injections of growth hormone and of desiccated thyroid for a period of two months stimulated a significant rise in blood glucose of 40 mg. % \( (P < .01) \). Injections of insulin resulted in a decrease in blood glucose of 25 mg. % \( (P = .05) \). Blood glucose levels of animals injected with a combination of growth hormone, desiccated thyroid, and insulin were identical with blood glucose levels of water injected controls.

Liver and Carcass Glycogen. The amount of glycogen found in livers of animals injected with a combination of growth hormone, thyroid, and insulin was only half the amount found in livers of control animals \( (P = .05) \). Liver glycogen was decreased slightly in growth hormone
injected animals and increased in thyroid and in insulin injected
animals. Differences in liver glycogen between experimental and control
animals did not prove to be statistically significant (P > .05).
No differences in carcass glycogen between control and experimental
animals were observed.
DISCUSSION

It is postulated that environmental stimuli, especially the photoperiod, influence growth and related seasonal changes in metabolism through the mediation of hormonal secretions. To test this hypothesis experiments were designed: (1) to determine whether hormones could promote growth in anoles "functionally hypophysectomized" by exposure to short photoperiods, (2) to further quantitate seasonal changes in nitrogen metabolism and to determine the effects of growth hormone and glucose on nitrogen metabolism and, (3) to further quantitate seasonal changes in carbohydrate metabolism and to determine the effects of various hormones on carbohydrate metabolism.

HORMONAL REPLACEMENT EXPERIMENT:

The inhibitory effect of short photoperiods on growth in anoles reported by Fox and Dessauer (1957) was confirmed by data on the two control groups of this experiment. No significant increase in length occurred in either summer or winter control groups. Weight increase in the summer controls can be attributed to lipid deposited during the first week of the experiment. During this period caloric intake was still elevated, 60 cal./g./day, although the mean for the full two month period was 40 cal./g./day. Fat deposition and decline in appetite are characteristic of the autumn anole (Dessauer, 1955b). Our data suggest
that in its response to an abrupt change from a summer photoperiod to a winter photoperiod there is a brief interval during which anoles resemble autumn animals.

For purpose of this discussion somatic growth may be defined as the increase in mass resulting from an increase in mineral and protein content, and excludes increase in mass due to deposition of fat. By this definition bovine growth hormone stimulated growth in "functionally hypophysectomized" anoles. Desiccated thyroid injection resulted in increased body weight but without significant increase in snout-vent length (presumably bone synthesis). Simultaneous injections of growth hormone and desiccated thyroid were not as effective as growth hormone alone. Insulin alone was without effect in increasing either weight or length. Although simultaneous injections of growth hormone, desiccated thyroid, and insulin were no more effective than growth hormone alone in stimulating increase in snout-vent length, their effectiveness in stimulating increase in protein was a summation of the effectiveness of growth hormone alone and thyroid alone. Growth hormone was the principal growth promoting substance of the compounds tested, its effect being augmented by thyroid powder and insulin.

Response to hormonal stimulation of growth differed from response to photoperiodic stimulation in that appetite was not augmented by the hormones used. Photoperiodic stimulation of appetite was, therefore, mediated either by other hormones or by some extra-hypophyseal mechanism. Growth obtained in hormone injected groups appeared to result from a more efficient utilization of food. The slightly greater maximal growth obtained with photoperiodic stimulation could be attributed to the simultaneous stimulation of both appetite and hormone secretion so
that the greater food intake was utilized with the same high efficiency as in animals injected with growth hormone, thyroid powder or a combination of growth hormone, thyroid powder, and insulin.

Marked taxonomic specificity in the response to bovine growth hormone has been reported. Bovine growth hormone is active in the cow, pig, sheep, dog, cat, rabbit, rat, and mouse, but not in the guinea pig or in any primate species including man (Smith, et. al., 1955; Russell and Wilhelmi, 1958). On the basis of elevation of blood glucose and, in combination with hydrocortisone, the production of a diabetic state, bovine growth hormone is also active in the alligator (Stevenson, et. al., 1957). The lizard, Anolis carolinensis, can be added to the list of species in which bovine growth hormone is active.

Table VII summarizes the activity and purity of bovine growth hormone as assayed in Anolis.

**TABLE VII**

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Criterion</th>
<th>Activity</th>
<th>References</th>
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</thead>
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<tr>
<td>Growth</td>
<td>Increase in body weight</td>
<td>+</td>
<td>Chapter II</td>
</tr>
<tr>
<td></td>
<td>Increase in bone length</td>
<td>+</td>
<td>Chapter II</td>
</tr>
<tr>
<td></td>
<td>Effect on nitrogen metabolism</td>
<td>+</td>
<td>Chapter II and III</td>
</tr>
<tr>
<td></td>
<td>Elevation of blood glucose</td>
<td>+</td>
<td>Chapter IV</td>
</tr>
<tr>
<td></td>
<td>Reduction of ratio of fat to</td>
<td>+</td>
<td>Chapter II</td>
</tr>
<tr>
<td></td>
<td>body weight</td>
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<tr>
<td>ACTH</td>
<td>Expansion of melanophores</td>
<td>-</td>
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<tr>
<td>MSH</td>
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<tr>
<td>FSH</td>
<td>Testis Weight</td>
<td>-</td>
<td>Footnote 2</td>
</tr>
</tbody>
</table>

1. Injections of ACTH in dosages as small as $10^{-10}$ mg. into anoles results in an immediate general expansion of melanophores. The effect lasts for several hours. Injections of growth hormone produced only a slight discoloration at the injection sites.

2. No differences in testis weight between control animals and growth hormone injected animals of the hormonal replacement experiment were detected.
NITROGEN METABOLISM STUDIES:

Nitrogen excretion in both fasting and fed anoles was maximal in spring and summer and minimal in autumn and early winter. Cyclic changes in blood amino nitrogen, maximal in February and minimal in autumn, were slightly out of phase with changes in nitrogen excretion (Dessauer, personal communication). Such data suggest that in Anolis nitrogen metabolism was more active during the Growth and Reproduction period than during other seasons.

Seasonal differences in nitrogen metabolism appear to have a positive survival value in adaptation to the environment. In spring and early summer, when lipid and carbohydrate content of Anolis is minimal, fasting nitrogen excretion is maximal. The average fasting nitrogen excretion at this season indicates that Anolis obtains 62% of its standard metabolic requirement by burning its tissue proteins. At least 20% of its tissue protein would be catabolized in a fast of ten days. Body protein can contribute calories to meet standard metabolic requirements for a limited time only. Nearly half of the animals brought into the laboratory in spring died during the four day fast preceding experiments. However, in spring the food supply of insects is high and anoles are normally feeding. Associated with increased food supply and consumption there is an increased ability to utilize dietary nitrogen.

In contrast, food supply and voluntary food consumption of Anolis is minimal in late autumn and winter. However, during this period the lipid and carbohydrate deposited during the Storage period supply calories to meet standard metabolic requirements. Average fasting nitrogen excretion indicates very little protein catabolism even at 28°C.
The nitrogen excretion rate of Anolis in October indicates that protein catabolism supplies only 20% of the standard metabolic requirements and represents a loss of only 7% of its tissue protein during a ten day fast. In Anolis, a poikilotherm, the standard metabolic rate at 18°C, is only half the rate at 28°C. (Dessauer, personal communication). Assuming a proportional decrease in the rate of protein catabolism, it would take 20 days for Anolis to lose 7% of its tissue protein at natural winter temperatures. Even if daytime temperatures were to be elevated, as often occurs in Louisiana in winter, protein destruction, as measured by nitrogen excretion, would be low enough to permit survival. Mortality rates observed in the laboratory were very low in winter, even at elevated temperatures. Data on feeding experiments indicate that in winter not only was the ability to excrete nitrogen from endogenous sources diminished, but also the ability to excrete nitrogen from exogenous sources. Due to the limitations of the experiments, the disposition of this dietary nitrogen could not be ascertained.

In late summer and autumn synthesis and retention of lipid and carbohydrate must be proceeding at a rapid rate judging from the change in composition of the animal from August to October. The normal diet of Anolis consists mainly of insects which, assuming the composition of meal worms is representative, is presumably low in carbohydrates (Mellanby, 1932). The hormonal and enzymatic systems operating during this period must favor conversion of glucogenic amino acids to glucose and synthesis of glycogen from this newly formed glucose. The occurrence of "obligatory" glycogenesis, coupled with an increased ability to metabolize fats to supply a greater fraction of maintenance calories could explain in part the secondary peak in nitrogen observed in autumn.
In October, when tissue glycogen was at a maximum, fasting nitrogen excretion was at a minimum. Furthermore, as carbohydrate stores diminished during late autumn, winter, and early spring, fasting excretion progressively increased. The apparent relationship between carbohydrate content and fasting nitrogen excretion is further suggested by data on glucose feeding. In February 25 mg. glucose "spared" 14.8 mg. of tissue protein during the three day collection period or 0.59 mg. protein/mg. glucose. In July and August 0.44 mg. of tissue protein was spared per milligram glucose. Furthermore, nitrogen excretion was still reduced four to six days after feeding glucose. Glucose sparing ratios obtained on other species by adding glucose to diets adequate in energy content were: man - 0.04 (Cuthbertson, et al., 1937; Cuthbertson and Munro, 1937), dog - 0.11 (Iarson and Chaikoff, 1937), and rat - 0.09 (Forbes and Swift, 1944). In comparison, glucose is quite effective in spring protein in Anolis.

Not only were endogenous and exogenous nitrogen excretion elevated in Anolis during spring and summer but they were increased in all seasons with injections of growth hormone. However, Anolis does grow in spring and summer (Gordon, 1956), when exposed to long photoperiods equivalent to summer (Fox and Dessauer, 1957), or when injected with growth hormone over a long period of time (Chapter II). Amino acid nitrogen has two major routes of disposal - incorporation into tissue protein or excretion via the urine. The data on nitrogen excretion indicate that in spring an enzymatic and hormonal system exists whereby the rates of the involved reactions are accelerated. The evidence, although indirect, indicates that growth hormone may be one of these factors. Growth could occur under these conditions if protein intake
is sufficient to shift the equilibrium in favor of protein synthesis by a mass action effect. Anoles in which growth was stimulated by exposure to long photoperiods voluntarily consumed two to three times the amount of calories needed to fulfill standard metabolic requirements (Fox and Dessauer, 1957). The results obtained with growth and other hormones in long term experiments show that an increase in calorie consumption of only 60% above standard metabolic requirements can lead to growth. Similar experiments on other species have shown that growth hormones will promote growth only in animals which are well fed and in a good nutritional state (Smith, et. al., 1955; de Bodo and Altszuler, 1957; Russell and Wilhelmi, 1958). The negative nitrogen balance obtained after feeding in spring suggest that some deficiency in essential amino acids may have been incurred during the fasting period before feeding.

GLUCOSE METABOLISM EXPERIMENTS:

Glucose Disposition. Seasonal variations in the manner in which a 4.5 g. Anolis disposed of a load of 40 mg. glucose are summarized in Figure 11. For the greater part of the year tissue glycogen had reached or passed its maximum at 48 hours and blood glucose was approaching fasting levels. The figure depicts the disposition of the injected glucose at 48 hours. Glucose converted to glycogen was estimated by subtracting fasting absolute glycogen content from absolute glycogen content 48 hours after injection of glucose. Glucose concentration was considered to be equal in both blood and extracellular fluid. Extracellular fluid volume (glucose space) was estimated as 20% of the body weight (Dessauer, 1952). Glucose retained in the extracellular fluid was calculated by multiplying the extracellular fluid volume by the
difference between fasting and 48 hour blood glucose levels.

In September and October approximately half of the injected glucose was accounted for as liver glycogen and 10% appeared in the urine. The undetermined portion, assumed to be oxidized or converted to other organic metabolites, approached a minimum in late October. In early winter a maximum, 70% of the injected glucose, was converted to tissue glycogen. Marked changes occurred in the partition of glycogen. From early November to March disposal of glucose as liver glycogen progressively decreased while disposal as carcass glycogen increased to a maximum in December, then slowly decreased. During this season the undetermined ("oxidized") glucose, blood glucose, and urinary glucose also increased. Furthermore, carcass glycogen began to disappear 48 to 96 hours after glucose injection. These findings indicate that in winter glucose was rapidly mobilized to extra-hepatic tissues where utilization of glucose as an energy source was proceeding rapidly. In the Growth and Reproduction period of spring and summer "oxidation" of glucose was maximal, ability to synthesize glycogen was minimal, and more glucose was lost by excretion than at other seasons.

Taking into account the entire balance of glucose disposal and especially considering urinary loss, utilization of glucose is maximum in autumn and winter, and minimum in summer. If only the rate of disappearance of blood glucose, as in a classical glucose tolerance test, were used as a measure of the efficiency of an animal to utilize glucose, then the opposite conclusion would be drawn. In carbohydrate metabolism studies in which an animal is given large loads of glucose, the fate of the glucose injected may not be clearly estimated by conventional tolerance tests.
Seasonal variations in gross tissue composition, similar to those observed in Anolis, occur in a wide variety of vertebrate species. Cyclic alterations in fasting blood glucose levels occur in the frog (Smith, 1949), alligator (Coulson, et al., 1950), turtle (Lopes, et al., 1954), and duck (Miahle, 1958). Seasonal differences in carbohydrate metabolism as measured by the classical glucose tolerance test has been observed in the laboratory rat (Cori and Cori, 1927), turtle (Lopes, et al., 1954), and duck (Miahle, 1958). Pre-winter storage of fats and carbohydrates occur in amphibians (Athanasiu, 1899; Smith, 1949), birds (Wolfson, 1959) and in hibernating and non-hibernating mammals, including man (Sargent, 1954).

Effects of Hormones on Tissue Carbohydrates - Blood Glucose. The striking rise in blood glucose stimulated by glucagon injections and the high blood glucose levels of lizards when compared to vertebrates other than birds (Dessauer and Fox, 1959; Miller and Wurster, 1958), suggest that lizards are very sensitive to glucagon. Miller demonstrated the preponderance of alpha cells, presumably the pancreatic cells which secrete glucagon, in the lizard, Eumeces obsoletus. The chicken (Hazelwood and Lorenz, 1957) and the duck (Miahle, 1958), species which also have high fasting blood glucose levels, are also sensitive to glucagon. Total pancreatectomy in the duck (Miahle, 1958) and in the lizard, Eumeces obsoletus, (Miller and Wurster, 1959) results in a hypoglycemic state rather than the classical diabetes observed in mammals.

Injections of 1 unit/kg. of insulin into an alligator (Coulson and Hernandez, 1953) and turtle (Lopes, et al., 1954) resulted in marked decreases in blood glucose after an initial rise (due to contamination with glucagon). Injections of the same dosage into a snake (Prado, 1947)
and into anoles (Dessauer, personal communication) resulted in an initial rise in blood glucose but did not significantly reduce blood glucose below the pre-injection levels. Dosages of 500 to 1000 units/kg. caused an initial rise in blood glucose followed by a later fall in blood glucose of the order of 20-30% below pre-injection levels in the duck (Mahle, 1958) and the snake, Bothrops jararaca (Prado, 1947) as well as Anolis (Table V and VI). In Anolis injected with 500 units (25 mg.)/kg. of insulin daily for 60 days, blood glucose was again only about 15% less than that of control animals.

The commercial method used in preparation of bovine insulin in America does not completely eliminate glucagon contamination. Thus in careful studies in mammals and in all thorough studies in poikilothermic vertebrates, an initial rise in blood glucose is always obtained before the expected decrease in blood glucose. However, in Anolis injected with 500 units/kg. of a specially prepared, glucagon-free insulin, blood glucose levels were again only slightly lower than controls, further indicating an insensitivity to bovine insulin.

The activity of bovine glucagon in Anolis is conservatively estimated to be twenty times greater than the activity of insulin. Injections of 1 mg./kg. glucagon abolished the effects on blood glucose of 25 mg./kg. simultaneous injection of glucagon-free insulin and resulted in a net rise in blood glucose level.

It appears that animals of the line of vertebrate evolution that includes birds, lizards, and snakes are insensitive to bovine insulin but quite sensitive to bovine glucagon.

The injection of thyroid, epinephrine, growth hormone, and a combination of growth hormone and hydrocortisone caused an increase in
blood glucose in \textit{Anolis} just as in other species (de Bodo and Altszuler, 1958).

**Effects of Hormones on Tissue Glycogen.** Glucagon injections in \textit{Anolis}, as in other species (Foa, \textit{et al.}, 1957), produced a decrease in liver glycogen and had no effect on carcass glycogen. In accordance with the findings of Sutherland, Woslait, and Rall (1956), that the primary effect of glucagon is the activation of inactive liver phosphorylase, the magnitude of rise in blood glucose was correlated with initial liver glycogen levels. The high liver glycogen and its extreme stability in autumn clearly indicates that glucagon secretion is greatly reduced or absent in the Storage period.

Injections of either commercial insulin or glucagon-free insulin resulted in decreased liver glycogen and increased carcass glycogen. Although a wealth of data indicate the same effects in other species, De Duve (1956, 1960) maintains that decrease in liver glycogen is not produced by insulin itself but by glucagon contamination even in so-called glucagon-free insulin preparations. This may be correct since in \textit{Anolis}, a species sensitive to glucagon, injections of a combination of glucagon and glucagon-free insulin magnified the changes in tissue glycogen produced by insulin alone. The preferential disposition of glucose into the carcass rather than into the liver of \textit{Anolis} in winter indicates that both glucagon and insulin secretion are elevated in the Hibernation period.

The effects of epinephrine and thyroid powder on tissue glycogen of \textit{Anolis} were no different than the effects obtained in other species (de Bodo and Altszuler, 1958). The results obtained with thyroid powder were inconclusive. Epinephrine injections produced a decrease
in both liver and carcass glycogen. In short term experiments growth hormone injections consistently produced a decrease in carcass glycogen. In earlier experiments growth hormone was reported to retard the rate of glycogen breakdown in carcasses of fasting hypophysectomized rats, the glycostatic effect of growth hormone (Russell, 1938; Russell and Wilhelmi, 1950), and increase carcass glycogen in fasting normal rats (Illingworth and Russell, 1951). Recently, however, carcass glycogen in fasting normal rats was reported to be unaffected by growth hormone (Russell and Bloom, 1956). At this time no definite conclusions can be made regarding a general interspecific effect of growth hormone on carcass glycogen.

**TISSUE CARBOHYDRATES IN THE HORMONAL REPLACEMENT EXPERIMENT:**

Blood glucose levels in control animals were significantly higher than in insulin injected animals and significantly lower than in either growth hormone or thyroid injected animals. In Anolis injected with a combination of growth hormone, desiccated thyroid, and insulin, the hyperglycemic effects of growth hormone and thyroid were counterbalanced by the hypoglycemic effect of insulin. Significant differences in liver glycogen between control and experimental animals were obtained only with the combination of growth hormone, desiccated thyroid, and insulin. Liver glycogen of control animals was high, as in the Storage period of autumn, whereas liver glycogen of experimental animals was low, as in the Growth and Reproduction period of spring and summer.
SUMMARY

Optimal growth was obtained in "functionally hypophysectomized" anoles injected with a combination of bovine growth hormone, desiccated thyroid, and insulin. The glucagon contaminant in the insulin may have been necessary for optimal growth. Unlike exposure to long photoperiods, which stimulate growth with increased calorie consumption, hormone injections stimulated growth without augmentation of appetite. Growth of hormone injected animals appeared to result from more efficient utilization of food.

Nitrogen metabolism was active in spring and summer when protein was utilized as an energy source and in growth processes. Nitrogen metabolism was decreased in winter when food consumption and growth processes were minimal, and maintenance calories were obtained from catabolism of stored fats and carbohydrates. Injections of growth hormone increased nitrogen metabolism.

Injected glucose was converted to liver glycogen in autumn, carcass glycogen in winter, and was oxidized in spring and summer. Anolis was sensitive to glucagon and insensitive to insulin. Glucagon injections resulted in a decrease in liver glycogen. Glucagon and insulin acted synergistically in the transportation of glucose from liver to muscle. Growth hormone injections produced a hyperglycemia but,
contrary to findings in other species, led to a decrease in extrahepatic glycogen. Tissue carbohydrates in "functionally hypophysectomized" anoles injected with a combination of growth hormone, desiccated thyroid, and insulin was low as in anoles during spring and summer.

The hypothesis is advanced that seasonal differences in the physiological state of Anolis appear to be controlled by cyclic changes in activities of its endocrine organs. Growth occurs only when endocrine secretions are in favorable balance.
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Title of Thesis: "Hormonal regulation of growth in the lizard, Anolis carolinensis"

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Date of Examination:

January 10, 1961