

ting time. A clotting defect was demonstrated in recalcified oxalated plasmas of burros tested 2 weeks after irradiation. 3. All irradiated burros developed a severe thrombocytopenia and a lessening of clot retraction was noted on the after 4th post irradiation day, with no clot retraction after the 16th day when the platelet count was approaching zero. 4. There was a pronounced diminution of prothrombin utilization rate after irradiation. The first change was noted 22 hours after irradiation or 3 hours after the animals were removed from the exposure field. 5. There was no demonstrable circulating anticoagulant. The possible relation of platelet concentration to the clotting defects is discussed.

1. Cronkite, E. P., Brecher, George, *Ann. Rev. Med.*, 1952, v3, 193.
2. Trum, B. F., Rust, J. H., and Wilding, J. L., *Auburn Vet.*, 1952, v8, 131.
3. Wilding, J. L., Simons, C. S., and Rust, J. H., *Nucleonics*, 1952, v10, 36.
4. Rust, J. H., Trum, B. F., Wilding, J. L., and Simons, C. S., *Radiology*, in press.
5. Wintrobe, M. M., *Clinical Hematology*, Lea & Febiger, Philadelphia, 1946, 2nd Edition, 193.
6. Quick, A. J., and Stefanini, M., *J. Lab. and Clin. Med.*, 1949, v34, 973.
7. Rosenfield, R. E., and Tuft, H. S., *Am. J. Clin. Path.*, 1947, v17, 405.
8. Ware, A. G., and Seegers, W. H., *Am. J. Clin. Path.*, 1949, v19, 471.
9. Buckwalter, J. A., Blythe, W. F., and Brinkhous, K. M., *Am. J. Physiol.*, 1949, v159, 316.
10. Langdell, R. D., Graham, J. B., and Brinkhous, K. M., *Proc. Soc. Exp. Biol. and Med.*, 1950, v74, 424.
11. Ferguson, J. H., Andrews, G. A., and Brucer, M., *Proc. Soc. Exp. Biol. and Med.*, 1952, v80, 541.
12. LeRoy, G. V., *Arch. Int. Med.*, 1950, v86, 691.
13. Cronkite, E. P., *Blood*, 1950, v5(1), 32-45.
14. Penick, G. D., Cronkite, E. P., Godwin, I. D., and Brinkhous, K. M., *Proc. Soc. Exp. Biol. and Med.*, 1951, v78, 732.
15. Jackson, D. P., Cronkite, E. P., Jacobs, G. J., and Berhens, C. F., *Am. J. Physiol.*, 1952, v169, 208.
16. LeRoy, G. V., and Halpern, B., *J. Lab. and Clin. Med.*, 1950, v39, 449.
17. Cohn, S. H., *Blood*, 1952, v7, 225.
18. Rosenthal, R. L., and Benedek, A. L., *Am. J. Physiol.*, 1950, v171, 505.
19. Tocantins, L. M., Carrol, R. T., and Holborn, R. H., *Blood*, 1951, v6, 720.
20. Tocantins, L. M., *Am. J. Physiol.*, 1936, v114, 709.
21. Quick, A. J., Shonberg, J. N., and Stefanini, M., *Am. J. Med. Science*, 1949, v217, 198.
22. Dillard, G. H. L., Brecher, Geo., and Cronkite, E. P., *Proc. Soc. Exp. Biol. and Med.*, 1951, v78, 796.
23. Allen, J. G., Moulder, P. V., and Emerson, D. M., *J.A.M.A.*, 1951, v145, 704.
24. Cronkite, E. P., Jacobs, J. G., Brecher, G., and Dillard, G., *Am. J. Roentgenol.*, 1952, v67, 796.
25. Alexander, B., and Landwehr, G., *J. Clin. Invest.*, 1949, v28, 1511.
26. Ferguson, J. H., *Trans. 5th Conference Josiah Macy, Jr., Foundation, N. Y.*, 1952, 268.

Received January 19, 1953. P.S.E.B.M., 1953, v82.

Hibernation of the Lizard, *Anolis carolinensis*. (20114)

HERBERT C. DESSAUER. (Introduced by Roland A. Coulson.)

From the Department of Biochemistry, Louisiana State University Medical School, New Orleans, La.

Any attempt to evaluate the comparative biochemistry of hibernation is hindered by the sparsity of information on seasonal changes among reptiles. As part of an extensive study of reptilian biochemistry, analyses of certain metabolic indices were done each month on the lizard, *Anolis carolinensis*, to compare the effect of the seasons on animals obtained from

the wild with groups kept under laboratory conditions.

In order to determine whether seasonal temperature changes are primarily responsible for the metabolic variations found, a colony of captive lizards was maintained in a windowless, constant temperature room at the average summer temperature of New Orleans,

28 C. It was divided into 2 groups so that the effect of light could be checked. Since south Louisiana receives 14 hours of sunlight in June and 10 hours in December, these groups were exposed to light for 10 and 14 hours respectively. All animals were acclimatized for at least 3 weeks before being used in any experimental procedure. By the end of 3 months the colony was totally depleted by the frequent sacrifice of animals for analyses and required restocking. Every 4 days the animals were fed meal worms, the larval form of the insect, *Tenebrio molitor*. Since *Anolis* will not drink from a dish but acquires its water from drops on leaves, ferns and ivy were kept in the cages during the investigation and their foliage was generously sprinkled with water every day.

Analytical routine. Fifty freshly caught animals were sacrificed each month along with 50 animals from each of the 2 captive groups. Twenty of these animals from each group were used for blood glucose and liver studies, 20 for estimation of total ether extractives (lipid) and 10 for respiration determinations. All animals were fasted 4 days prior to use since it was found that all traces of food disappear from the digestive tract within this period. The animals used for glucose and liver studies were weighed, decapitated and 0.05 to 0.10 ml of available blood was measured for determination of blood glucose(1). The carcass was opened quickly down the midline, the liver was removed, weighed and immediately dropped into boiling 30% KOH solution for subsequent glycogen analysis(2). Animals used for lipid analysis were dried to constant weight in a vacuum desiccator; the carcass was ground into fine pieces and extracted with ether. The extract was filtered, the ether evaporated and the lipid weighed. Respiration was studied in a closed space respiration chamber which consisted of a liter Erlenmeyer flask fitted by means of a 3 holed rubber stopper to a water manometer, thermometer and a pinch clamp for pressure adjustment. The animals were placed into sacks made of net cloth and conditioned in a darkened incubator at 28 C for 4 hours. The sacks were then suspended from the bottom of the rubber stopper of the manometer unit, and the entire

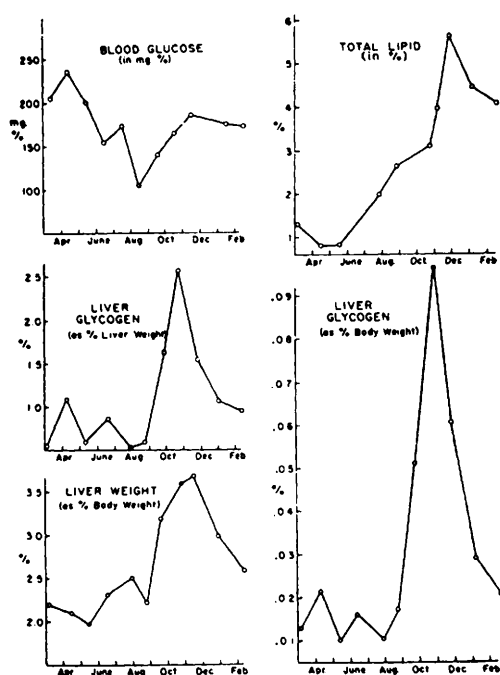


FIG. 1. Seasonal variations *Anolis carolinensis* (nature group).

unit was stoppered into an Erlenmeyer flask which contained 25 ml of standard $\text{Ba}(\text{OH})_2$ solution. The respirometer was placed into the darkened incubator. After an hour the manometer was adjusted to atmospheric pressure and temperature and barometer readings were recorded. Forty-four to 52 hours later readings of these same variables and the manometer level were taken, and the $\text{Ba}(\text{OH})_2$ was titrated with standard HCl. Oxygen utilization was calculated from the pressure, temperature and manometric changes and CO_2 production from the results of the titration.

Results. A composite picture of the seasonal changes found in animals received fresh from the wild is shown in Fig. 1. Blood glucose varies with the season. Both experimental groups and each sex had practically the same average blood sugar and exhibited the same seasonal periodicity as the controls from the wild.

Liver glycogen increases along with the weight of the liver in the fall. The calculation of liver glycogen as % body weight, therefore, presents a more accurate picture of this increased glycogen storage than does a graph of

% liver glycogen. Although all experimental groups exhibited the same changes as the controls, the livers of the captive animals were always slightly heavier and contained more glycogen. Extrahepatic glycogen also is elevated in the fall and low in the spring. Analyses in September and in February on carcasses remaining after routine liver studies on recently captured animals gave values of 229 and 63 mg/100 g body wt respectively.

In order to test the possibility of keeping the weight of the large livers of the fall animals up by feeding, a group of lizards was placed under dark colony conditions in November and their livers were analyzed in February. Although these animals were given food in the season when wild lizards have a scarcity of food, their livers decreased in weight and glycogen in the same manner as those recently captured.

The ether extractable substances of the dried animal carcass reach a low value in late spring and then increase progressively, reaching a maximum in the month of November after which a gradual decline is noted. Captive animals exhibited the same seasonal periodicity as the control animals from the wild state.

There are no marked seasonal variations in the O₂ utilization or CO₂ production of *Anolis*. All changes from the mean are of the order of 10 to 15%. From March through June O₂ utilization, measured at 28°C, averages 0.198 ± 0.0027* ml/g body wt/hr, whereas during the remainder of the year it averages 0.220 ± 0.0027* ml/g body wt/hr. Although the mean RQ is high (0.914 ± 0.0034*) in all seasons, the lowest individual values recorded occurred in April in animals fresh from the wild.

Discussion. Using standard caloric values for fat and carbohydrate, one can calculate from the data presented that the reserve energy of *Anolis* reaches a peak of about 55 kcal/100 g body wt in the fall and drops to about 10 kcal/100 g body wt in the spring. Only

3% of this reserve energy is due to carbohydrate.

No similar quantitative work on seasonal liver studies on lizards or other reptiles could be found in the literature. Beginning with the work of Athanasia(3), however, many workers have observed seasonal changes in amphibians. Livers of the frog are larger and have an increased energy content in the fall as compared with other seasons. Blood sugar varies seasonally in the alligator(4) also but remains constant throughout the year in the snakes, *Bathrops jararaca* and *Philodryas sp* (5). If the O₂ consumption of reptiles is measured at the same temperature in different seasons there are no marked changes. *Anolis carolinensis*, *Alligator mississippiensis*(4), and *Storeria dekayi*, a snake(6) have seasonal differences of less than 15%. In contrast to reptiles the frog, *Rana*, requires 50 to 70% more oxygen in the spring than in the winter (7).

Summary. In late summer and fall, body weight and to a greater extent liver weight increase due to the storage of glycogen and fat. With the increased storage of liver glycogen the blood glucose falls to a low level. In the winter and spring the blood sugar rises to a maximum and the liver glycogen and total lipids fall as the animal uses its body stores. Neither varying the hours of light to which the animals were exposed nor keeping them at a constant temperature throughout the year had any marked effect upon the constituents analyzed.

1. Folin, O., and Wu, H., *J. Biol. Chem.*, 1920, v41, 367.
2. Good, C. A., Kramer, H., and Somogyi, M., *J. Biol. Chem.*, 1933, v100, 485.
3. Athanasia J., *Arch. für Physiol. von Pflüger*, 1899, v74, 561.
4. Hernandez, T., and Coulson, R. A., *Proc. Soc. Exp. Biol. and Med.*, 1952, v79, 145.
5. Prado, J. L., *Memorias do Instituto do Butantan*, Butantan, Brazil, 1946, v19, 59.
6. Clausen, H. J., *J. Cell. and Comp. Physiol.*, 1936, v8, 367.
7. *Tabulae Biologicae*, W. Junk, Berlin, 1926, v3.

Received January 15, 1953. P.S.E.B.M., 1953, v82.

$$* P.E. = 0.6745 \sqrt{\frac{(\sum x^2)}{n(n-1)}}$$