Comp. Biochem. Physiol. Vol. 60B. pp. 491 to 493.
© Pergamon Press Ltd 1978. Printed in Great Britain
0305-0491/78/0715-0491$02.00/0

THE INFLUENCE OF SEASON ON GLYCOGEN LEVELS IN THE LIZARD *LACERTA VIVIPARA* JACQUIN

J. W. PATTERSON[1], P. M. C. DAVIES[1], D. A. VEASEY[2] and J. R. GRIFFITHS[2]

[1] Department of Zoology, University of Nottingham, University Park, Nottingham NG7 2RD, England
[2] Department of Biochemistry and Chemistry, The Medical College of St. Bartholomew's Hospital, Charterhouse Square, London EC1M 6BQ, England

(*Received* 20 *September* 1977)

Abstract—1. Quantities of glycogen in liver and muscle tissue of *Lacerta vivipara* were determined at different times of year.

2. Glycogen content of muscle is less in summer than in spring and autumn.

3. Glycogen content of liver is low in spring and early summer but increases progressively in late summer and autumn.

4. Glycogen accounts for only 2–3% of the energy store of *Lacerta vivipara* entering hibernation.

INTRODUCTION

Many terrestrial ectotherms hibernate in temperate regions. Differences in the way of life of active and hibernating animals and the preparation necessary for hibernation result in considerable seasonal variation in a number of bodily functions (Gilles-Baillen, 1974). In particular, because feeding is not possible during hibernation, hibernators must store considerable quantities of energy-yielding materials in the preceding period. There is now a considerable literature which demonstrates that lipid is stored by vertebrates before hibernation and used as an energy source during hibernation (see reviews by Derickson, 1976, and Fitzpatrick, 1976).

Stored carbohydrate has received less attention, although it has been demonstrated that glycogen content varies seasonally in much the same way as lipid in the lizards *Anolis carolinensis* (Dessauer, 1953) and *Egernia cunninghami* (Barwick & Bryant, 1966) and the frog *Rana temporaria* (Pasanen & Koskela, 1974). In this last species, glycogen forms 40–50% of the energy reserves of frogs entering hibernation (Pasanen & Koskela, 1974).

This paper reports a study on seasonal variation in liver and muscle glycogen content in the lizard *Lacerta vivipara*, a cool-temperate species which hibernates for several months each year (Smith, 1964).

MATERIALS AND METHOD

All *Lacerta vivipara* used in this study were adults; the snout–vent length of the lizards varied between 45 and 64 mm.

Lacerta vivipara were collected from two sites in the south of England. Attempts were made to collect both male and female lizards on every occasion, although this was not always possible. Lizards were normally killed within 24 hr of capture, although in some cases they were maintained for up to 2 weeks in an outside vivarium at Nottingham before being killed.

Lizards were killed approximately at monthly intervals during the active season (late March to early October). In addition, a group of lizards which had been hibernated artificially in a constant temperature room at Nottingham since the previous October was killed in early March shortly before the lizards were due to emerge from hibernation.

Lizards were killed by placing them in a deep freeze at −25°C. Thereafter they were stored at this temperature until required for glycogen estimation, at which time they were removed from the deep freeze, thawed, and dissected. 10 mg–100 mg of both liver and muscle tissue was removed, weighed and transferred to a centrifuge tube for glycogen determination.

Glycogen was estimated in muscle and liver by the method of Seifter *et al.* (1950), modified by the addition of a drop of 10% (w/v) sodium sulphate solution to the sodium hydroxide digest before precipitating the glycogen with ethanol. This acted as a co-precipitant and improved glycogen recovery, particularly with dilute samples. The early results obtained without using this co-precipitant have been corrected for the glycogen lost during the ethanol precipitation step. Glycogen losses were found (in a series of control experiments with muscle extracts) to vary linearly between 5 μg in an extract containing 20 μg and 9 μg in an extract containing 100 μg. The early results were corrected using the linear interpolation program supplied with the Monroe 1806 calculator. None of these corrections significantly affected the conclusions drawn from the results.

RESULTS

In no case could any significant difference in glycogen levels be detected between males and females. For convenience, therefore, the results from the two sexes have been pooled. Concentrations of glycogen in muscle and liver (mg glycogen·g wet weight tissue^{-1}) are shown in Fig. 1 and 2. Figure 3 shows the liver glycogen concentrations in terms of mg glycogen·g wet weight whole lizard^{-1}; the muscle glycogen results have not been expressed in this fashion because the total muscle mass of the animals was unknown.

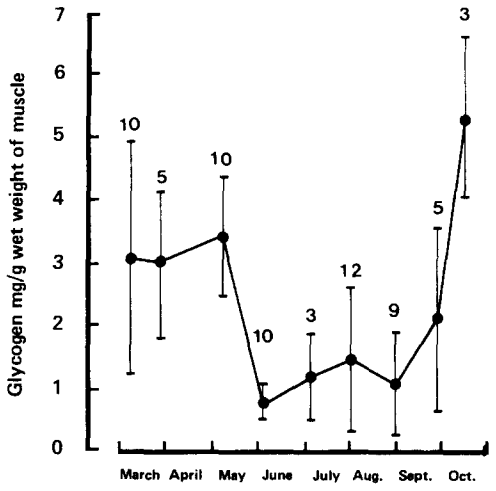

Fig. 1. Seasonal variation in glycogen content of *Lacerta vivipara* muscle. The points and error bars represent means and standard deviations and the numbers indicate the lizards in each group.

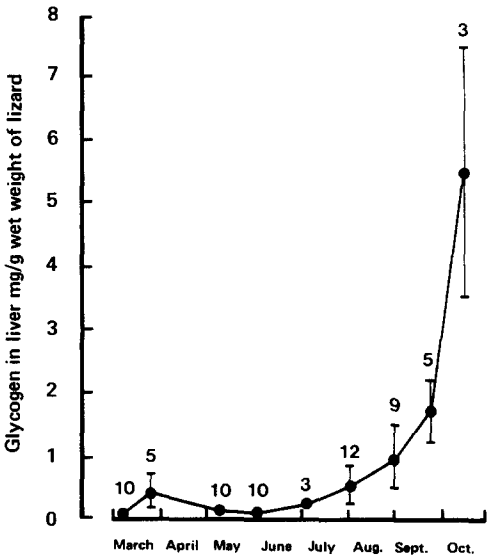

Fig. 3. Seasonal variation in liver glycogen content of *Lacerta vivipara*. Symbols as in Fig. 1.

Quantities of glycogen stored in both muscle and liver showed considerable variation; lizards caught and killed at the same time frequently had glycogen contents which varied by as much as an order of magnitude. Since the lizards show so much within-group variation, it is unwise to employ any of the common parametric tests to analyse this data. Therefore the Mann–Whitney *U*-test (a non-parametric test) has been used for all analyses.

Glycogen concentration in muscle varied between 0.1 and 7 $mg \cdot g^{-1}$ muscle. Some seasonal variation could be detected, for summer (June–August) values were significantly lower ($P = <0.01$) than spring (March–May) and sutumn (September, October) values.

Glycogen concentration in the liver shows very marked seasonal variation. The glycogen content is low near the end of hibernation (early March) and during the early months of the active season, but from the beginning of July to the time the lizards enter hibernation in October there is a build up of liver glycogen. The glycogen content of the liver in the latter half of the season (July–October) was significantly higher ($P = <0.01$) than early on in the year (March–June). Thus there is an increase in liver glycogen in the late summer and autumn, and *Lacerta vivipara* enters hibernation with more glycogen in its liver than at any other time of year.

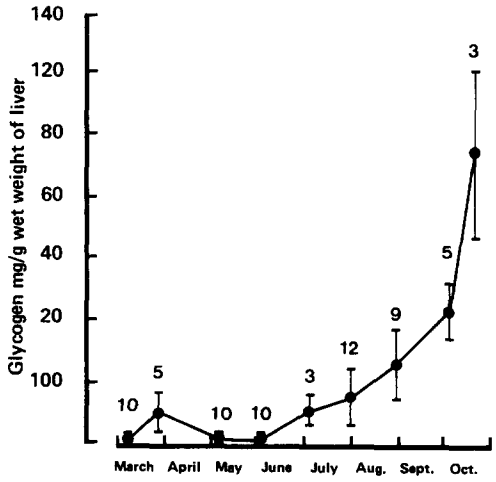

Fig. 2. Seasonal variation in glycogen content of *Lacerta vivipara* liver. Symbols as in Fig. 1.

DISCUSSION

Although muscle glycogen content shows a less marked seasonal variation than does liver glycogen content, the levels of glycogen in muscle in June, July and August are less than at other times of year. This may reflect greater muscular activity in the warmer months of the year.

From the data in Avery (1970, 1971), it is possible to calculate that an adult *Lacerta vivipara* has about 5.1 $kJ \cdot g^{-1}$ stored as lipid just before entry into hibernation. The figures presented here demonstrate that about 0.10 $kJ \cdot g^{-1}$ is stored as glycogen in the liver of *Lacerta vivipara* just prior to hibernation (these figures are calculated on the assumption that lipid contains 39 and glycogen 17 $kJ \cdot g^{-1}$; Magnus-Levy, 1907; Passmore & Draper, 1965). Thus liver glycogen stores account for less than 2% of the total stored energy of an adult *Lacerta vivipara* about to enter hibernation. Even if muscle glycogen is included (assuming that muscle constitutes 50% of body weight, as in crocodiles—Coulson & Hernandez, 1974), glycogen forms less than 3% of the total stored energy. In this respect *Lacerta vivipara* is similar to the lizard *Anolis carolinensis*, in which energy in the form of carbohydrate accounts for about 3% of the total energy store (Dessauer, 1953).

About 1.8 $kJ \cdot g^{-1}$ are used in metabolism during hibernation by adult *Lacerta vivipara* (Patterson & Davies, unpublished observations). Since a total of only 0.13 $kJ \cdot g^{-1}$ are stored as glycogen (0.10 $kJ \cdot g^{-1}$ in liver, 0.03 $kJ \cdot g^{-1}$ in muscle), even if the whole of the glycogen store were expended during hibernation, less than 10% of the energy required during hiberna-

tion could be obtained from glycogen. Thus *Lacerta vivipara* differs sharply from the frog *Rana temporaria* (another cool-temperate ectotherm which hibernates for several months each year) in which glycogen accounts for 40–50% of the energy store just before hibernation and 20–30% of the energy utilised during hibernation (Pasanen & Koskela, 1974).

Nevertheless, the quantity of glycogen in the liver of *L. vivipara* increases markedly in the late summer and autumn. In this respect *L. vivipara* is similar to *Anolis carolinensis*, which also shows a considerable increase in liver glycogen in the autumn (Dessauer, 1953), although the actual quantities of glycogen stored (about 5.6 mg·g body weight^{-1} in *L. vivipara*, 1 mg·g body weight^{-1} in *A. carolinensis*) are greater in *L. vivipara*.

Although the quantity of glycogen in the liver is too small to be a significant source of energy during hibernation it could act as an important source of glucosyl residues. These could be formed during hibernation from three main sources: liver glycogen, the glycerol component of glyceride fats and by gluconeogenesis from carbon skeletons of amino acids. Maximizing the glucose produced from the first two sources would minimize the protein breakdown necessary to supply the third. The quantities of glucose available from the lipid and liver glycogen consumed during hibernation may be calculated from the data of Avery (1970, 1971), Avery *et al.* (1974) and those in the present paper. The liver glycogen of a 3 g lizard is depleted by approximately 18 mg during hibernation; this would give 20 mg glucose when hydrolysed. The lipid stores of *L. vivipara* are depleted by approximately 200 mg during hibernation (Avery, 1970, 1971). Since this lipid is 90% triglyceride and 4–9% diglyceride, and fatty acid constituents are 75% C_{18} and 20% C_{16} (Avery *et al.*, 1974), this 200 mg should give about 22 mg of glycerol which would form almost the same weight of glucose. Thus the glyceride glycerol and liver glycogen could each supply about 20 mg glucose during hibernation.

Acknowledgements—We thank Miss R. Connell and Miss E. A. Wilkie for technical assistance and Professor B. Ryman for suggesting the modified method of glycogen precipitation. This work was supported in part by a grant from the Science Research Council.

REFERENCES

AVERY R. A. (1970) Utilization of caudal fat by hibernating common lizards, *Lacerta vivipara*. *Comp. Biochem. Physiol.* **37**, 119–121.

AVERY R. A. (1971) Estimates of food consumption by the lizard *Lacerta vivipara* Jacquin. *J. Anim. Ecol.* **40**, 351–365.

AVERY R. A., SHEWRY D. R. & STOBART A. K. (1974) A comparison of lipids from the fat body and tail of the common lizard, *Lacerta vivipara*. *Br. J. Herpet.* **5**, 410–412.

BARWICK R. E. & BRYANT C. (1966) Physiological and biochemical aspects of hibernation in the scincid lizard *Egernia cunninghami* (Gray, 1832). *Physiol. Zool.* **39**, 1–20.

COULSON R. A. & HERNANDEZ T. (1974) Intermediary metabolism of reptiles. In *Chemical Zoology* (Edited by FLORKIN M. & SCHEER B. T.), Vol. 9, pp. 217–247. Academic Press, New York.

DERICKSON W. K. (1976) Lipid storage and utilization in reptiles. *Am. J. Zool.* **16**, 711–723.

DESSAUER H. C. (1953) Hibernation of the lizard, *Anolis carolinensis*. *Proc. Soc. exp. Biol. Med.* **82**, 351–353.

FITZPATRICK L. C. (1976) Life history patterns of storage and utilization of lipids for energy in amphibians. *Am. Zool.* **16**, 725–732.

GILLES-BAILLEN M. (1974) Seasonal variations in reptiles. In *Chemical Zoology* (Edited by FLORKIN M. & SCHEER B. T.), Vol. 9, pp. 353–376. Academic Press, New York.

MAGNUS-LEVY A. (1907) In *Metabolism and Practical Medicine* (Edited by NORDEM C. VON), Vol. 1, p. 190. Heinemann, London.

PASANEN S. & KOSKELA P. (1974) Seasonal and age variation in the metabolism of the common frog, *Rana temporaria* L. in northern Finland. *Comp. Biochem. Physiol.* **47A**, 635–654.

PASSMORE R. & DRAPER M. H. (1965) Energy metabolism. In *Newer Methods of Nutritional Biochemistry* (Edited by ALBANESE A. A.), pp. 41–83. Academic Press, New York.

SEIFTER S., DAYTON S., NOVIC B. & MUNTWYLER E. (1950) The estimation of glycogen with the anthrone reagent. *Archs Biochem.* **25**, 191–200.

SMITH M. A. (1964) *The British Amphibians and Reptiles*, 3rd edn. Collins, London.