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Seasonal variations in liver metabolism of the green frog *Rana esculenta* (L.)

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Summary. Rana esculenta (L.) kept under natural conditions show almost constant b.wt in the annual cycle. Liver weight, however, has a distinct peak in October/November which is also evident in the liver index. The liver storage materials: glycogen, triglyceride and protein, show 2 distinct maxima (spring, autumn).

There are only a few experimental data concerning the seasonal variations of metabolism in Amphibia[1-8]. Less attention was given to natural environmental conditions in some works concerning several aspects of these problems in frogs. Also experiments mostly were not carried out in a uniform population. Therefore, in the present paper, experiments are described which were performed during an annual cycle with a population of *Rana esculenta* (L.) living under natural conditions as far as possible.

In Amphibia fat body and liver represent 2 important organs which are able to store depot substances. Therefore the liver is the preferable organ for experiments concerning anabolism, catabolism and conversion of storage material.

Experimental animals and methods. Rana esculenta (L.) obtained from a commercial dealer served as experimental animals. They were kept outdoor 2 months before experiments started. The open-air grounds (3 × 8 m) consisted of a land area with natural vegetation, stones and sticks and a water compartment with continuous flow. The frogs were fed additionally to naturally offered food with mealworms and flyblows.

Every month a random sample of 10 animals was taken from this population. After decapitation, the liver was removed and homogenized. It was stored at −20 °C until further elaboration. Determination of liver protein was carried out according to[9] with a modified Biuret-method. Liver glycogen values were obtained according to[10] with a modified o-Toluidin-method. Determination of liver triglycerides were made with the test kit Ingotest 567651 (Boehringer)[11]. The data were compared by the t-test according to student.

Results. The b. wt of the experimental animals is relatively constant 60–70 g (figure, A) whereas the liver weight shows a seasonal rhythm: it increases significantly from September on and is not positively correlated to the b. wt (Aug./Sept., Aug./Oct., $p < 0.001$). Because of slight variations of the b. wt in spring, seasonal variations of the liver weight are less distinctive (figure, A).

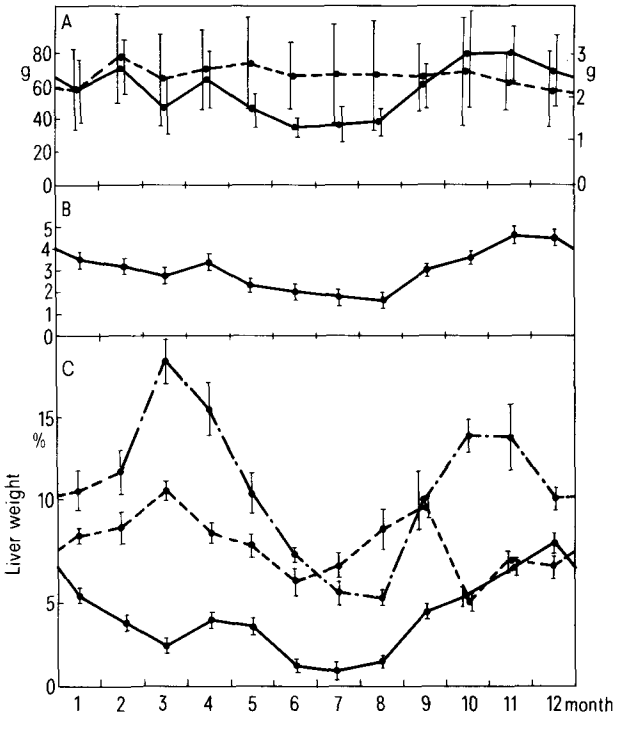
The liver index established with both parameters is a 1st aspect of the metabolic situation of the liver: it shows a distinct increase in September with a maximum in November (Aug./Sept., Aug./Nov., $p < 0.001$). From this time on a continuous decrease until August is observed, which is interrupted only by a less distinct spring maximum in April (figure, B) (March/April, April/May, $p < 0.001$). The liver protein values of the experimental frogs show 2 maxima in the annual cycle: a spring peak in March and a late summer maximum in September (figure, C) (Feb./Mar., March/April, $p < 0.001$; July/Sept., Sept./Nov., $p < 0.001$).

The content of glycogen in the liver shows a clear maximum in December and a less distinct one in April (Mar./Apr., April/May, $p < 0.001$; Aug., Nov./Dec., Dec./Jan., $p < 0.001$). The lowest glycogen values are determined in the summer months June, July, August. From this time on, the rapid increase towards the December maximum begins, which then decreases to the March minimum. This is followed by the spring maximum (figure, C). The liver triglyceride values also show a distinct seasonal rhythm: an autumn maximum (October, November) is accompanied by a spring maximum in March (Aug./Oct., Nov./Dec., $p < 0.001$; Feb./Mar., Mar./Apr., $p < 0.001$).

The lowest values are obtained during the summer months July and August (figure, C).

Discussion. These results show that in *Rana esculenta* (L) the storage material in the liver decreases from November/December which is characteristic for the 4-month winter phase. During this time and in the following spawning period, the frog does not take any food. Therefore a sufficient amount of depot substances has to be built up in summer and autumn. Thus the b. wt of the frogs should be reduced in the winter months. In the present experiments, however, this effect was not observed (figure, A). In *Rana temporaria* there is even an increase of b. wt in winter[12]. In *Rana esculenta,* in the months before spawning, a slight increase of body water content is observed[13]. This water uptake may explain the constant b. wt of the experimental animals. The increase of liver weight before spawning is caused by an intensified deposition of storage material in this organ (figure, A), which is certainly connected with an increase of water content in the liver[13]. The same is evident for the increase of liver weight before entering the winter phase (figure, A). These increases of water and storage material in the liver takes place parallel and may be pointed out as an increased metabolic activity of this organ. Also the protein content of the liver shows a distinct seasonal rhythm. Although protein certainly is not a suitable substrate for energy supply, it might play an important role in energy requirements during starvation. In this way, it becomes quite understandable that all substrates investigated show 2 concentration maxima. The spring maximum certainly supports the spawning phase. The 2nd maximum before entering the winter period may be explained by the synthesis of depot substances for the fasting months.

Whereas the spring maxima of protein and triglycerides are found in March, the glycogen maximum is shifted by 1 month into April. A possible explanation for this phenomenon may be that the depot substances accomplish their main function in processes which are differing in time. Triglycerides and protein could be involved in anabolic processes directed to the spawning phase, whereas glycogen might serve as energy supply for spawning s.str. Also the 2nd maximum of liver storage material shows temporal differences. Whereas the protein maximum is found extremely early in September, the glycogen maximum occurs in December. The highest values for triglycerides are observed between those peaks. The late glycogen maximum in December shows evidence for the reason of the temporal differences in depot substances maxima. It is impossible that the December maximum is the direct effect of food intake because there is no more food consumption during this period. The increase of glycogen concentration could be caused by a rearrangement of glycogen from other organs e.g. muscle. This is very improbable, however, because in *Rana temporaria* e.g. the glycogen contents in muscle reach their highest values at this time[14]. The glycogen peak may be explained much more probably by a conversion from other depot substances such as triglycerides. In contrast to the glycogen maximum, the triglyceride peak (October, November) seems to be a direct effect of food intake, which then ceases in *Rana esculenta* at this time. The storage of protein in the liver serves as a protein depot on 1 hand, and as the initial point for gluconeogenesis on the other. The effectivity of the gluconeogenesis may be described by the activity of some enzymes. Results are reported on phosphoenolpyruvate carboxykinase in *Rana catesbeiana*[15] and glucose-6-phosphatase in *Rana esculenta*[16]. Both enzymes show the characteristic feature that their activity is very low in winter and reaches their highest values in July, August and September. This induces the effect that protein incorporated into the liver, far above the necessity for depot formation, can be strongly converted into glucose and at last into glycogen. In this way, the protein peak in September may be explained.



Seasonal variations of body weight, liver weight (A), liver index (B), liver glycogen, liver triglyceride and liver protein (C) in *Rana esculenta* (L). Each point represents the mean value of 10 animals. Vertical bars represent SE. Abscissae: months of the year. Ordinates: *A* left: body weight (g), right: liver weight (g). - - - -, body weight; ———, liver weight.

B liver index $\frac{\text{liver weight} \times 100}{\text{body weight}}$.

C - - - -, liver protein; ———, liver glycogen; -·-·-·-, liver triglyceride.

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