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

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Summary. Rana esculenta (L.), kept under natural conditions, show almost constant body weights in the annual cycle. Fat body weight, however, has a distinct maximum in October decreasing continuously to a May minimum which is also evident in the fat body index. The triglyceride and protein contents show the same course. Surprizingly high amounts of glycogen are found, which reach a maximum in August.

The fat body of the Amphibians represents a storage organ which is of importance for life in the sub-oceanic climatic zone of central and northern Europe. Published results only deal with the total lipid contents of the fat body[1-5]. Triglycerides represent the primary form of energy storage, and for this reason the present paper makes an attempt to determine the triglycerides in this organ. Furthermore, we intended to test whether or not the fat body has further functions in the Amphibian metabolism. In this context, it is very important that the results reported in this paper are gained from a population of experimental animals as far as possible living under natural and uniform conditions.

Experimental animals and methods. Rana esculenta (L.) obtained from a commercial dealer served as experimental animals. They were kept outdoors for 2 months before experiments started. The open-air ground (3 × 8 m) consisted of a land area with natural vegetation, stones, sticks and a water area with continuous flow. In addition to the natural foods in their surroundings, the frogs were fed with mealworms and flyblows.

Every month a random sample of 10 animals was taken from this population. After decapitation of the frogs, the fat body was removed and homogenized. It was stored at −20 °C until further use. Determination of fat body protein was carried out according to Weichselbaum[6] with a modified Biuret-method. Fat body glycogen values were obtained according to Bertram and Otto[7] with a modified o-Toluidin-method. Determination of fat body triglycerides was made with the test kit Ingotest 567651 (Boehringer)[8]. The data were compared by the Student t-test.

Results. The fat body weight shows a distinct seasonal rhythm, whereas the total body weight of the frogs is relatively constant (figure A). The fat body weight significantly increases from June and reaches its maximum in October; thereafter it decreases continuously until the May minimum (Sept./Oct., Oct./Nov., $p < 0.01$ and $p < 0.001$ respectively).

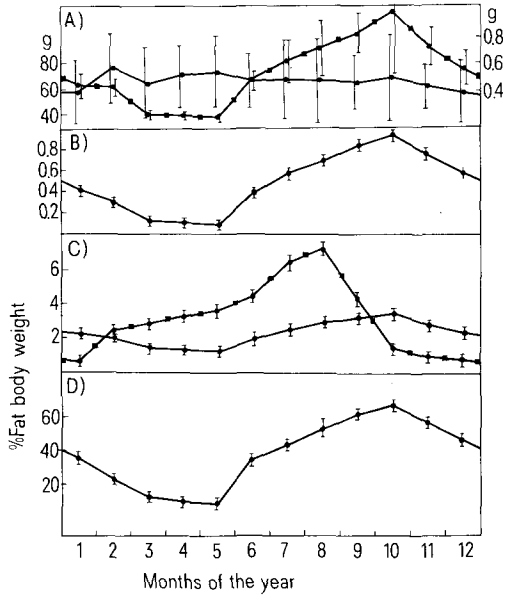
The fat body index obtained from the 2 parameters clearly shows that the fat body weight is not correlated with the body weight in the annual cycle (figure B). The fat body index also shows this seasonal rhythm with a distinct increase from June to a maximum in October, followed by a continuous decrease to its minimum is reached in May (Sept./Oct., Oct./Nov. $p < 0.001$). The triglyceride content of the fat body shows the following seasonal differences (figure D): a steep increase from June to the October peak, and from then on a winter decrease to the minimum in May (Sept./Oct., Oct./Nov., $p < 0.001$). In general the protein content of the fat body is very low (figure C). Nevertheless there are seasonal differences: from June onwards there is a definite increase to an October maximum followed by a decrease until the May minimum (Sept./Oct., Oct./Nov., $p < 0.001$).

The glycogen content in the fat body is minimal in the time from October to January (figure C). From February onwards there is a continuous increase ending in an August peak (July/Aug., Aug./Sept., $p < 0.001$). This slowly synthesized supply is quickly consumed in the following months until October.

Discussion. The results reported in this paper show the general tendency that, with the exception of 1 parameter, all others examined in the fat body reach their maximum in October and drop to a minimum during winter.

The fat body weight shows a significant annual cycle (figure A). The drastic decrease in the fat body weight starting in October demonstrates that the loss of weight caused by the release of storage substances during winter is never balanced by a corresponding water intake. Especially the fat body index (figure B) shows that the weight of this organ is not proportional to the body weight. The physiological function of the fat body in frogs therefore seems to be limited to the synthesis of a sufficient energy depot for hibernation.

The annual rhythm of this organ is clearly determined by its main depot product, the triglycerides (figure D). Weight changes seem to be the direct result of corresponding triglyceride release and intake. The concentration of this substance is directly related to the regeneration of the fat body early in the annual cycle. This result is supported by the course of the fat body weight curve. The beginning of



Seasonal variations of body weight, fat body weight (A), fat body index (B), fat body glycogen, fat body protein (C), fat body triglyceride (D) 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: fat body weight (g), *B* fat body index (fat body weight × 100)/body weight. Graphs: *A* -------, body weight, ■------■, fat body weight. *C* ■------■, fat body glycogen; --------, fat body protein.

the active period in *Rana esculenta* corresponds with the beginning of the fat body renewal. These results are in contrast to the storage of depot substances in the liver, which begins later[9]. However, the triglyceride reaches its maximum in October in the fat body as well as in the liver. The continuous decrease in the fat body's triglyceride content from October to May clearly shows the decisive role of this depot organ for the energy supply during the winter fasting period of *Rana esculenta*. The distinct decrease in the fat body triglyceride ends in March. At that time, this substrate reaches its second maximum in the liver[9]. That means that at least at this time triglycerides derived from the fat body are accumulated in the liver. It is possible that they are converted or incorporated in greater complexes, taking into account the spawning season.

The protein content of the fat body undergoes a comparable annual rhythm to that of the triglycerides (figure C). The mass of the incorporated protein seems to be negligible, serving as an additional energy supply. It is more likely that the protein concentration is in direct correlation with the mechanism of triglyceride incorporation and release.

Surprisingly the fat body contains a relativly high amount of glycogen (figure C), which is too high to consider the fat body in *Rana esculenta* only as a triglyceride storage organ. This correlation strikingly resembles the brown adipose tissue in higher vertebrates. Because the glycogen maximum is found in August, in contrast to the triglyceride's in October, there could be different physiological demands as far as the energy supply is concerned. The glycogen increase in the fat body, however, seems to be less relevant in the period from February until May, because at this time the fat body weight strongly decreases. Although fat body weight increases in any case strongly from May by triglyceride incorporation, there is an additional glycogen level increase. In this correlation, it is specially striking that, during the fat body glycogen increase, the liver synthesizes no glycogen depot[9]. The liver glycogen does not increase before fat body glycogen decreases in September. The physiological background of this phenomenon can only be a subject of speculation at this time.

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Microbiological oxidation of the pentyl side chain of cannabinoids

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Summary. Syncephalastrum racemosum ATCC 18192 and *Mycobacterium rhodochrous* ATCC 19067 partially degrade the n-pentyl side chain of cannabidiol, cannabinol, Δ^8-tetrahydrocannabinol and Δ^9-tetrahydrocannabinol. Carboxylic acid and alcohol side chain derivatives are major metabolites.

We have screened more than 100 species of fungi and bacteria for the ability to transform 4 common cannabinoids, namely cannabidiol (CBD), cannabinol (CBN), Δ^8-tetrahydrocannabinol (Δ^8-THC) and Δ^9-tetrahydrocannabinol (Δ^9-THC). The 4 cannabinoids studied are known to possess a variety of potentially useful pharmacological activities in addition to the psychotropic effects for which *Cannabis sativa* L. and its preparations receive popular use[2]. Such physiological actions as anticonvulsant, antidepressant, hypotensive, bronchodilation and lowering of intraocular pressure have led a number of groups to investigate the possible development of useful medicinal agents from the growing ranks of naturally occurring and synthetic cannabinoids. A major emphasis in the study of the effects of these characteristic marihuana components on humans has been the elucidation of the routes of metabolism. In mammalian systems transformation reportedly proceeds by initial monohydroxylation at allylic positions in the monoterpene portion of the cannabinoid molecule or on the carbons of the aliphatic side chain. The subsequent introduction of additional hydroxyl groups and the further oxidation of some intermediate alcohols to aldehydes, ketones or carboxylic acids lead to a complex mixture of polar metabolites[3], which may be further complicated by conjugation. Because certain of these polar metabolites have not been produced in good yields by standard organic synthetic methods, we decided to investigate microbial transformation as a means of producing certain metabolites in quantities sufficient for animal testing and other studies[4]. In addition, some of the microbially catalyzed reactions may be useful tools in the preparation of novel synthetic cannabinoids.

Materials and methods. All screening and small scale production studies were carried out in shaken Erlenmeyer flasks (25 ml culture broth per 125-ml flask, or 100 ml per 500-ml flask) incubated at 25 °C and 250 rpm. The medium routinely used for the cultivation of the 2 organisms discussed in this study is a yeast-malt extract broth (Difco). Inoculation of sterilized medium is achieved by aseptic transfer of an aqueous suspension of surface growth (fungal conidia or bacterial cells) from agar slants into the culture flasks. After abundant growth is obtained (usually 24–48 h), the cannabinoid (obtained from the National Institute on Drug Abuse) was added as a small volume aliquot of a concentrated ethanolic solution, at a level of 10 or 20 mg/100 ml culture. Following suitable incubation periods, varying from 1 to 6 days, the cultures were adjusted to pH 3 and rapidly extracted with 3 equal portions of chloroform or ethyl acetate. Extracts containing cannabinoids and metabolites were dried over anhydrous sodium sulfate, concentrated under reduced pressure and examined by TLC[4].