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Gross Body Composition in Postnatal Development of the Bank Vole III. Estimating Age *

[With 2 Tables & 5 Figs.]

A statistical analysis of the relationship between age and water and protein content in the bodies of voles (*Clethrionomys glareolus*), and the index of these two parameters was made. Water and protein content were determined in voles kept in captivity for a considerable time, and in individuals born in captivity from parents caught under field conditions at the beginning and towards the end of the reproductive season. It was shown that both the parameters examined and the quotient of protein and water content are significantly correlated with age, and therefore these were used for estimating age, calculating the mathematical relations. The age of individuals is correlated to the highest degree with protein content, but this parameter depends on the season in which the animal was born. It is however possible to determine the age of a vole from water content during the first 3 months of its life, with an accuracy of ±14 days, irrespective of the season in which the animal was born.

I. INTRODUCTION

Age cannot be determined very accurately in rodents, as there are no characters changing with the individual's age which can be conveniently measured. The length of the molar roots has been taken as a criterion of age for *Clethrionomys glareolus*. This method was introduced by Zimmermann (1937), but growth of the molar roots begins after the tooth crowns have formed.

In later studies an endeavour has been made to determine accurately the age at which growth of the roots begins and also their rate of growth. It was, however, found that within the range of this species both the age

*) Praca została zrealizowana w ramach problemu węzłowego 09.1.7., koordynowanego przez Instytut Ekologii PAN.

at which tooth roots begin to grow and the rate of their growth vary (see Mazák, 1963; Pucek & Zejda, 1968; Viitala, 1971). It has also been shown that the rate of growth of tooth varies within a population an even within cohorts (*e. g.*, Claude, 1970; Lowe, 1971; Zejda, 1971), and even within one cohort it varies over the course of a year (Zejda, 1971). Even so, an account of the lack of more accurate methods, absolute age is determined with a accuracy of 1—2 months in *C. glareolus* on the basis of the formation of the neck of the tooth, the lenght of the molar roots and moult characters (Mazák, 1963; Tupikova, Sidorowa & Konovalova, 1968).

The relationship between gross body composition and age in rodents has been known for a long time (Moulton, 1923; Chanutin, 1931). Bender & Miller (1953) found that the ratio of the amount of nitrogen to the amount of water is a function of age in the rat. The mathematical relationship between this index of age and true age was used by them to estimate protein content on the basis of the water content found in individuals of a known age. When the changes in the proportions of gross body composition accompanying the development process in voles were analysed it was found that they are correlated with age (Fedyk, 1974 a, b). The purpose ot the present study is to ascertain whether the water and protein content of the voles' bodies are significantly correlated with age and whether age can be estimated on this basis.

II. MATERIAL AND METHODS

The water and protein content of 465 voles kept under laboratory conditions for many generations and in 193 individuals born at the beginning of the reproductive season (spring generation), and in 112 voles born at the end of the reproductive season (autumn generation) were measured using the methods described previously (Fedyk, 1974a). Gross body composition *(GBC)* was determined during the postnatal development of laboratory-bred voles over a period of 6 months, in the spring generation for 3 months and the autumn generation for 4 months of their life. The absolute age of all animals examined was known, as the voles of the seasonal generations formed the first generation born in captivity from wild parents caught at the beginning and before the end of the reproductive season.

The data obtained from the analyses were statistically elaborated by the straight line exponential regression methods calculating rate of increase in protein and water content and the quotient of these two components depending on age. The reverse relationships was also calculated, treating age as a function of the above basic components.

III. RESULTS AND DISCUSSION

From the data contained in Table 1 and those given in diagram form in Figs. 1—3 it can be seen that both water and protein content and

Table 1

Rate of quantative increase in water, protein and index of physiological age (*IPA*) (Y) depending on absolute age (X) in *C. glareolus*. Equations of straight-line and exponential regression together with their basic characteristic.

Groups	Y = water weight	Y = protein weight	Y = *IPA*
Spring generation (1—90 days)	$Y=+2.8144+.0807X$ $s_y=1.3286$; $sb=.0037$; $r=.843$ $Y=1.3065X^{.4317}$ $s_y=.0218$	$Y=+.5707+.0313X$ $s_y=.4719$; $sb=.0013$; $r=.863$ $Y=.1652X^{.6629}$ $s_y=.4040$	$Y=+.1982+.0020X$ $s_y=.0390$; $sb=.0001$; $r=.803$ $Y=.1303X^{.2235}$ $s_y=1.0597$
Autumn generation (1—120 days)	$Y=+2.8204+.0606X$ $s_y=1.4738$; $sb=.0036$; $r=.851$ $Y=1.4368X^{.3944}$ $s_y=1.024$	$Y=+.5071+.0215X$ $s_y=.0432$; $sb=.0010$; $r=.891$ $Y=.1619X^{.6198}$ $s_y=.3551$	$Y=+.1647+.0015X$ $s_y=.0393$; $sb=.0001$; $r=.837$ $Y=.1123X^{.2257}$ $s_y=.0204$
Laboratory (1—180 days)	$Y=+3.9654+.0403X$ $s_y=1.9078$; $sb=.0026$; $r=.696$ $Y=1.3808X^{.4050}$ $s_y=1.456$	$Y=+.9437+.0157X$ $s_y=1.0908$; $sb=.0026$; $r=.696$ $Y=.1629X^{.6349}$ $s_y=.6305$	$Y=+.2108+0010X$ $s_y=.0499$; $sb=.0001$; $r=.684$ $Y=.1181X^{.2292}$ $s_y=.0292$

also the index of these two parameter, are correlated with age. The regression equations contained in the table describing the above relations are significant with $P>0.001$. These relationships are of an exponential character during the first 30—40 days of life, and a straight line character during the subsequent period (see Figs. 1—3). For practical reasons the relationships between the body components and age have been described for the whole study period by one type of regression, but the exponential regression equations give a more accurate description of these connections than the straight line regression equations (cf. standard errors in estimation).

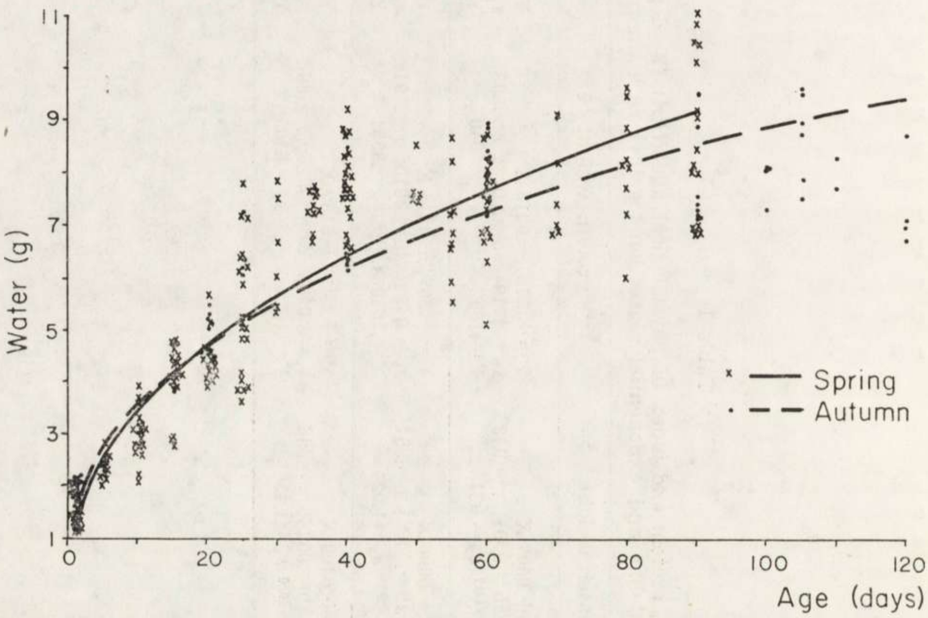

Fig. 1. Age—body water relation in the seasonal generations of *C. glareolus*.

As the age of an individual kept in captivity is always known, the data for this group of animals were included only for the sake of comparison. It was possible to show that despite almost uniform breeding conditions (Fedyk, 1974 a, b) statistically significant differences occur in the relationships between the parameters examined in the three groups of animals.

Water conent increases more quickly in voles of the spring generation than in those of the autumn generation and the variations in this parameter which were observed increase with age in both groups (Fig. 1). In voles born at the end of the reproductive season the coefficient of correlation for these characters is higher (respectively $r=0.843$,

$r = 0.851$). Water content in voles of different seasonal generations of comparable age is almost identical up to the 20th day of life, after which its growth is more rapid in the spring generation, and consequently on the 90th day of life of voles of the autumn generation there is 7.3% less water than in voles of the spring generation.

Protein is correlated with age to a greater degree than water ($r = 0.863$) and also increases more rapidly in the spring generation, Differences in the rate of increase for protein are greater than for water (Fig. 2).

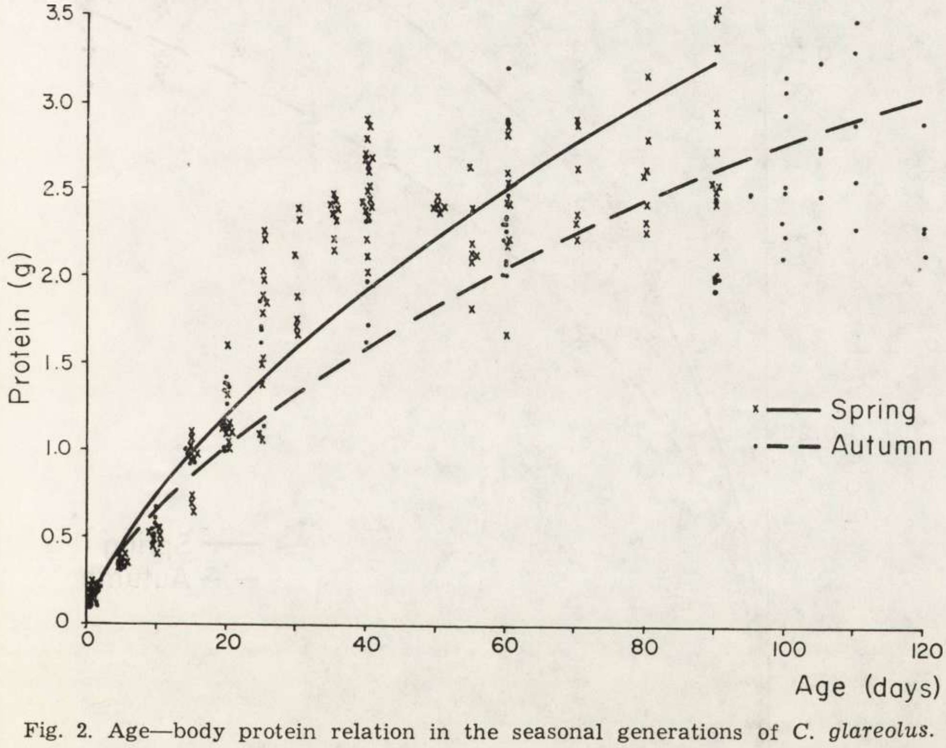

Fig. 2. Age—body protein relation in the seasonal generations of *C. glareolus*.

Voles of the spring generation have more protein even from the first days of life and this becomes more marked during growth, the difference being 18.9% on the 90th day of life. In laboratory-bred voles the rate of increase in water and protein content is intermediate in relation to the seasonal generations, as can be seen from comparisons of the relationships between these components and age calculated by means of exponential regression equations. On the basis of the straight line regression equations describing this relationship, however, it is the lowest of all the groups of voles compared. This contradiction is caused by the characteristics of a straight line regression and the fact that the studied period of development of the captive voles was twice as long as that of the seasonal generations. For the age interval from 3 to 6 months the value of the two body components increased at a far slower rate than during the first three months of life.

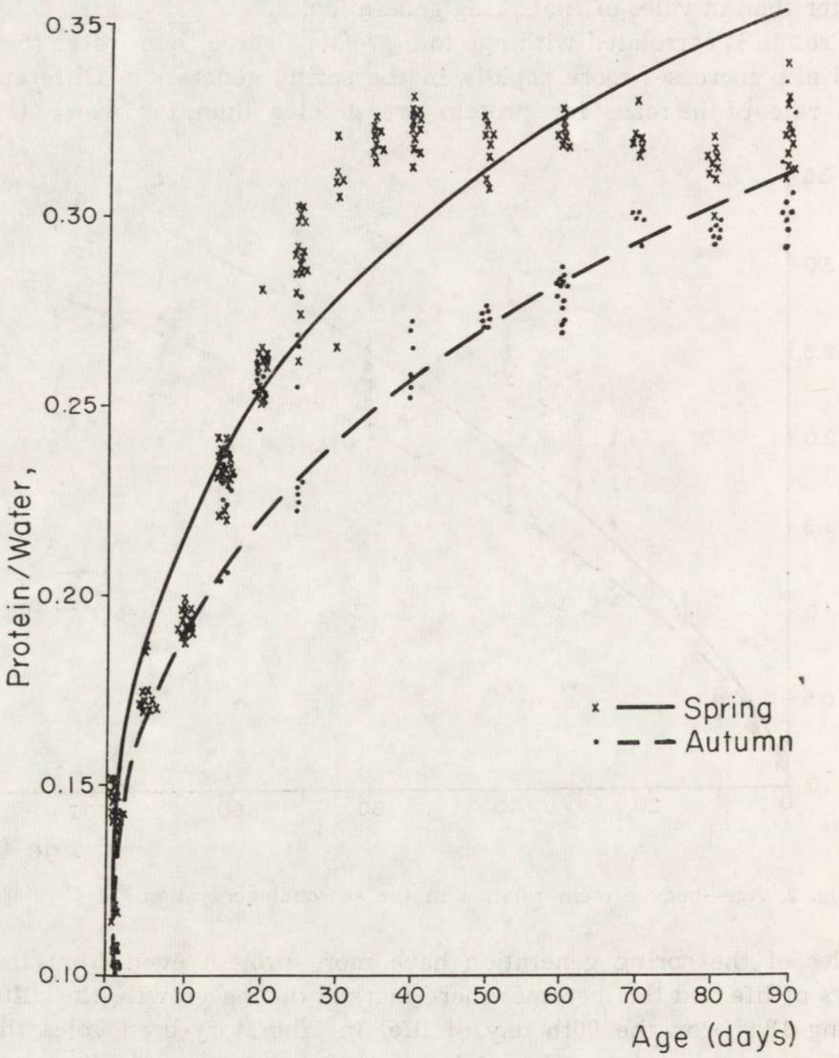

Fig. 3. Changes in protein: water ratio of seasonal generations of bank voles depending on age.

The quotient of the weight of protein and water, termed the index of physiological age (*IPA*) (B a i l e y, K i t t s & W o o d, 1960) is correlated to a lesser degree with age than protein and water. Like the

Table 2

Relation between age (X) and water and protein contents, and also index of physiological age (IPA) (Y) in *C. glareolus.*

Groups of voles	X = water weight	X = protein weight	X = IPA (protein to water ratio)
Spring generation	$Y = -15.2266 + 8.8113\,X$ $s_y = 13.88$; $sb = .406$; $r = .843$ $Y = +.8077\,X^{2.0580}$ $s_y = 14.53$	$Y = -\ 5.1729 + 23.8422\,X$ $s_y = 13.03$; $sb = 1.008$; $r = .863$ $Y = +15.7075\,X^{1.3211}$ $s_y = 12.97$	$Y = -51.0861 + 317.2248\,X$ $s_y = 15.40$; $sb = 17.06$; $r = .803$ $Y = +5358.2840\,X^{4.0851}$ $s_y = 14.13$
Autumn generation	$Y = -15.3869 + 8.2495\,X$ $s_y = 9.27$; $sb = .372$; $r = .924$ $Y = +.5621\,X^{2.2123}$ $s_y = 11.06$	$Y = -\ 6.0930 + 26.5510\,X$ $s_y = 9.04$; $sb = 1.136$; $r = .863$ $Y = +16.5541\,X^{1.4446}$ $s_y = 8.70$	$Y = -38.1005 + 296.0041\,X$ $s_y = 14.47$; $sb = 23.45$; $r = .803$ $Y = +6138.4778\,X^{3.8821}$ $s_y = 12.72$
Laboratory	$Y = -26.3293 + 12.8539\,X$ $s_y = 34.70$; $sb = .590$; $r = .730$ $Y = +.8800\,X^{2.0751}$ $s_y = 35.77$	$Y = -12.4125 + 36.2592\,X$ $s_y = 33.08$; $sb = 1.526$; $r = .569$ $Y = +18.6896\,X^{1.4034}$ $s_y = 33.65$	$Y = -83.3641 + 519.1509\,X$ $s_y = 36.66$; $sb = 26.53$; $r = .693$ $Y = +6803.5922\,X^{3.9733}$ $s_y = 35.44$

two parameters from which it was calculated, it increases far more quickly in voles of the spring generation than the autumn generation (Fig. 3). Differences in the physiological age of voles of the seasonal generations become greater during their lives, for instance while the eight-day old voles of the autumn generation correspond to approximately three-day old voles of the spring generation which have $IPA=0.180$. $IPA=0.300$ is characteristic of 42-day old individuals of the spring

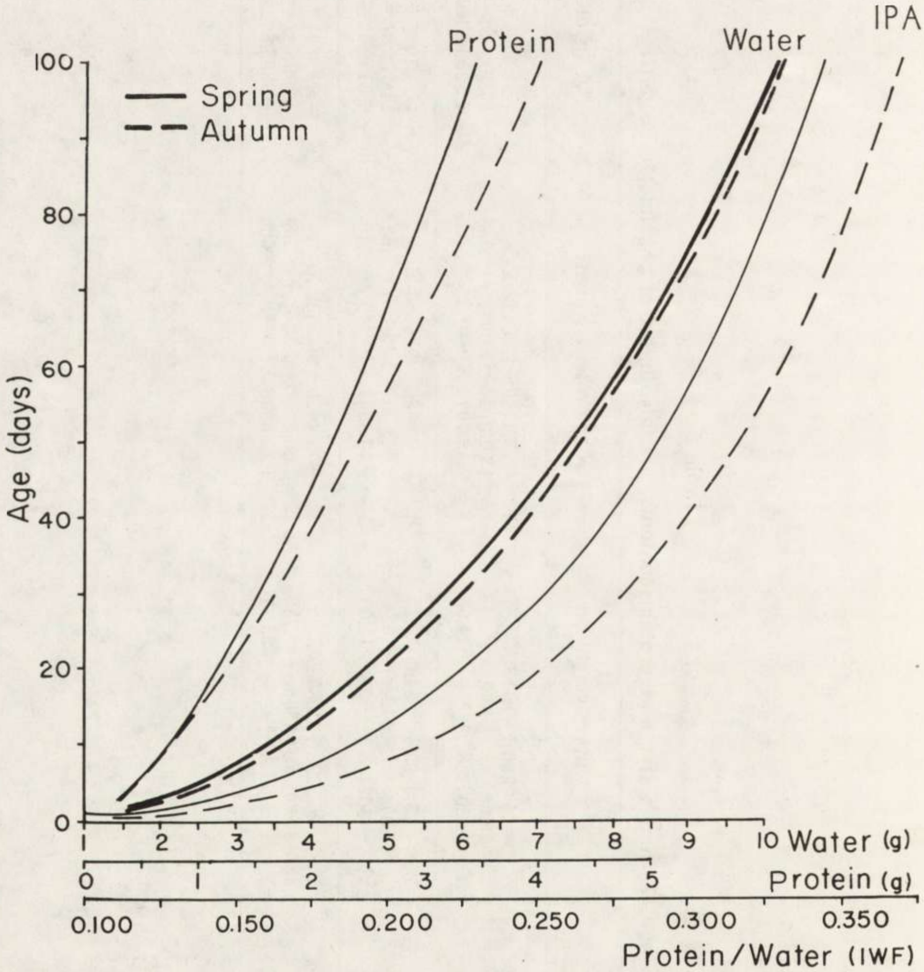

Fig. 5. Changes in body water, protein and physiological age with absolute age of bank voles of different seasonal generations.

generation and about 76-day old individuals of the autumn generation. On the 90th day of life the IPA of voles of the autumn generation is 12.4% lower than this index in voles of the spring generation.

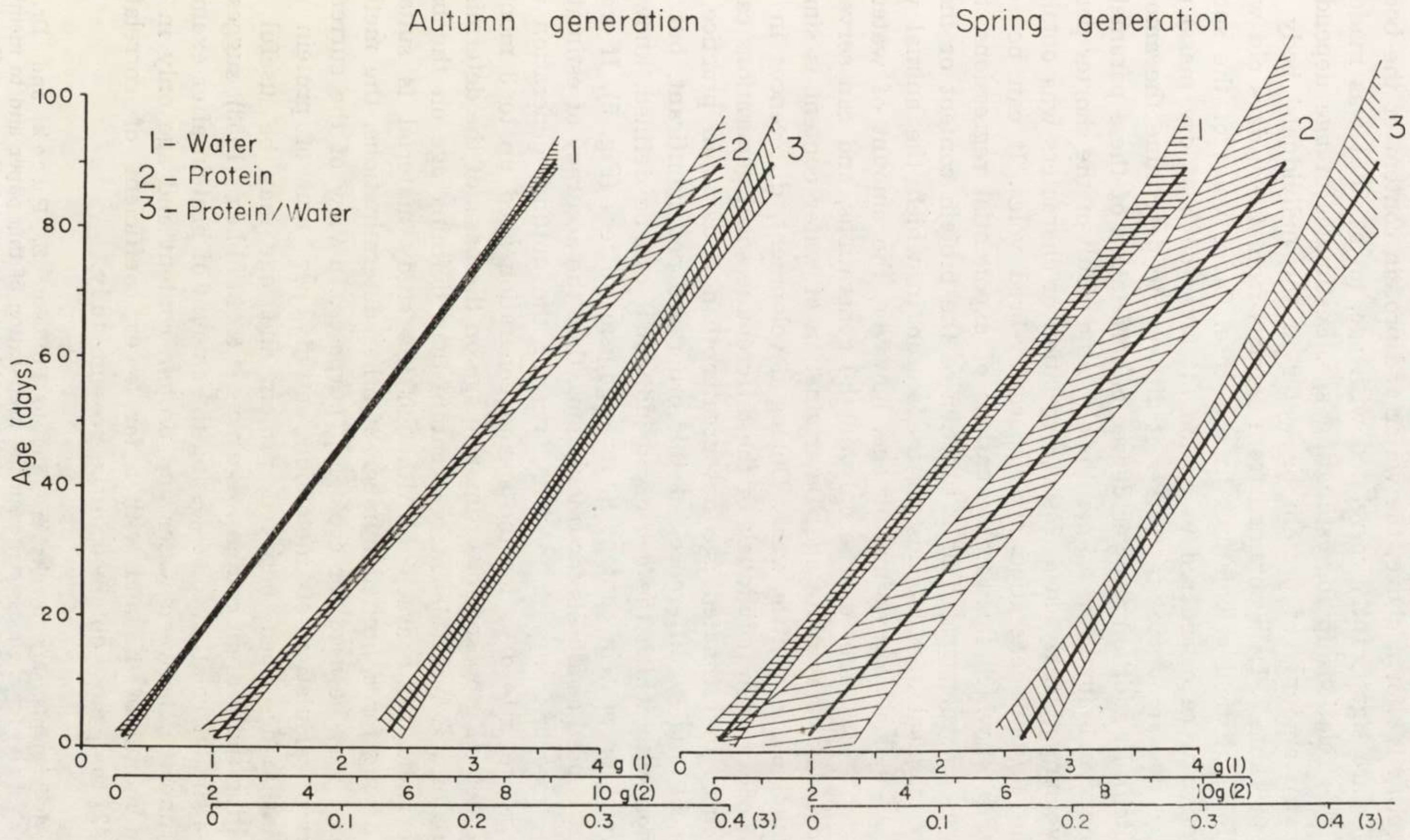

Fig. 5. Age of bank voles of seasonal generations depending on body protein (1), body water (2) and on physiological age (3).

Since, as is shown above, the water and protein content of the bodies of voles are significantly correlated with age, an attempt was made at using this relationship for estimating age. Eestimates of age depending on known amounts of protein or water in the individuals' body are given in Table 2. Data obtained from animals from 1—90 days old were used for statistical calculations in this case. Older voles of the spring generation were considered to be too fat (over 60% of dry mass) and an excessive amount of fat makes it difficult to determine the amount of protein. In addition a lower degree of correlation of these parameters was found in animals over 3 months old. As a result of the shorter period of development analyzed a closer correlation of characters was obtained for the autumn generation and laboratory-bred voles. It can be seen from Fig. 4, which ilustrates equations of exponential regressions, that the age of a vole treated as a function of the protein content or index of physiological age depends on the season in which the animal was born. As a basis for estimating age, however, the amount of water is dependent to a slight degree only on the generations, and can serve as a good index of absolute age. Determination of water content is simple and involves very little work. During development, differences in the weight of water in individuals of the different seasonal generations cause differences in estimated age not greater than 3 days. In practice, in ecological studies, differences of this order are not significant, but the precision with which the age of an individual can be defined, knowing the water or protein content in its organism, differs (Fig. 5). If water content is used as a basis for calculations, then the accuracy of estimation in this case is from ± 2 to ± 10 days for the autumn generation and from ± 7 to ± 14 days for the spring generation aged up to 3 months.

The method proposed for estimating age on the basis of the determined amount of water is thus more accurate than defining age on the basis of morphological characters. Although the study material is suitable after drying for a limited number of other determinations, the method proposed may be used for ecological purposes. In view of the currently preferred calculation of metabolic activity per unit of protein the relationship described between protein and age may be useful for estimating the protein content. Bender & Miller (1953) suggested using a similar relation for assessing the amount of protein after examing the connection between water and protein content and age only in the second month of life and with a far lower coefficient of correlation ($r = 0.42$) than those obtained in the present study.

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PODSTAWOWE SKŁADNIKI CIAŁA W ROZWOJU POSTNATALNYM NORNICY RUDEJ. III. SZACOWANIE WIEKU

Streszczenie

Poddano analizie statystycznej zależność między wiekiem a ilością wody i białka w organizmie nornic, *Clethrionomys glareolus* oraz ilorazem ilości białka i wody. Zawartość białka i wody oznaczono u nornic pochodzących z długoletniej hodowli oraz u osobników stanowiących pierwsze pokolenie urodzone w hodowli z dzikich rodziców odłowionych na początku i przed końcem sezonu rozrodczego. Wykazano, że zarówno zbadane parametry jak i ich iloraz (indeks wieku fizjologicznego, *IWF*) są skorelowane z wiekiem w sposób istotny (Tabela 1, Ryc. 1—3). Wobec tego zastosowano je do szacowania wieku, obliczając relacje między wiekiem a wymienionymi wyżej składnikami ciała (Tabela 2, Ryc. 4). Wiek osobnika najbardziej jest skorelowany z ilością białka lecz wielkość tego parametru zależy od sezonu w jakim się zwierzę urodziło. Natomiast z zawartości wody można oznaczyć wiek nornic w ciągu pierwszych trzech miesięcy życia z dokładnością do ±14 dni, bez względu na sezon, w którym się zwierzę urodziło (Ryc. 5). Opisana metoda oznaczania wieku u *C. glareolus* jest więc dokładniejsza od stosowanych dotychczas metod oznaczania wieku u tego gatunku według cech morfologicznych.