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Changes in the Gross Body Composition and the Caloric Value of the Common Voles during their Postnatal Development*

[With 4 Tables & 7 Figs.]

Changes in the chemical body composition were studied in 123 specimens of the common vole in the age between 0 and 30 days. The average body weight of voles increases with age (from 1.9 to 16.0 g). The percentages of water, protein and fat are highly correlated with body weight (r = 0.92). The water content in the body of voles decreases with age (from 83.7 to $63.3^{\circ}/_{\circ}$) similarly as the content of proteins (from 65.9 to $44.4^{\circ}/_{\circ}$). On the other hand the relative fat content increases (from 17.1 to $39.1^{\circ}/_{\circ}$), while the ash content remains fairly constant (around $9.5^{\circ}/_{\circ}$). In the fat-free biomass the water content decreases, while proteins and ash increase progressively with the age of animals. The energy values of the fat-free dry weight and ash-free dry weight remain on similar level. The index of physiological age expressed by the ratio of protein to water contents shows a rising trend with the body weight and age of voles.

I. INTRODUCTION

The knowledge of bioenergetic processes occurring in a population requires among others investigations of changes in the gross body composition of animals during their postnatal development. The growth of an animal is associated with one of the most important processes of the energy flow, namely the net production. The investigation of retention of particular components and of the energy constitutes an essential step in studying the dynamic changes of the energy value in the animal population.

Our knowledge of body composition, components of the body weight increase, as well as energetic values of growth and retention of particular components derives mainly from studies on farm and laboratory animals (Brody, 1945; Bailey *et al.*, 1960; Blaxter, 1962). Investigations of the whole picture of changes of the body composition during

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postnatal development of the common vole should provide more information on this phenomenon in wild rodents. Almost half of the total net production in the population of voles takes place during the foetal development and lactation (Petrusewicz & Walkowa, 1968).

II. MATERIAL AND METHODS

The contents of fat, protein, water and ash were analysed in the common vole, *Microtus arvalis* (Pallas, 1779), in the age between 0 and 30 days. The animals reared in the laboratory derived from the neighbourhood of Złotoryja $(51^{\circ}6' \text{ N}, 15^{\circ}54' \text{ E})^{-1}$). The first generation of voles bred in the laboratory and deriving from wild parents captured in natural habitats was used in the investigations. During the experiments the animals were supplied with food similar to the natural one (vegetables, corn, green forage).

Altogether 123 voles, divided into 7 age classes (0 - 1, 3, 6, 12, 20 and 30 days) were analysed (Table 1). In each class the gross body composition was determined in approximately 17 animals. The litters consisting of 4 to 6 specimens only were

Body composition (g)	Age in days						
	0 -1 (17)	3 (17)	6 (18)	9 (17)	12 (19)	20 (18)	30 (17)
Body weight	1.88	3.05	3.99	5.84	7.24	12.50	16.05
Water	1.57	2.39	3.00	4.16	5.12	8.51	10.19
Fat	0.05	0.20	0.32	0.55	0.70	1.39	2.29
Protein	0.20	0.38	0.54	0.87	1.07	2.05	2.60
Ash	0.04	0.06	0.09	0.15	0.19	0.37	0.53

Table 1.

Changes in gross body constituents of voles during the first month of their lives. In brackets are given numbers of examined individuals.

used for the analyses. From each litter 3 young voles of similar body weight were selected. One of them was employed for the determination of fat content, the second one for protein content, and the third one for the caloric value and ash content.

The animals were weighed and killed by the overdose of ether. The carcasses were cut open on the ventral side and dried in a vacuum oven at 60° C to a constant weight. The fat content was determined in a Soxhlet apparatus. A dried sample was put into the extraction thumble made of pressed blotting paper. The extraction was carried out with diethyl ether in 500 ml flasks placed in a water bath at 55°C. One carcass was extracted 30 times on the average with change of ether after 10 runs.

Nitrogen was determined by the Kjeldahl method. The protein content in the vole's body was calculated by multiplying the total nitrogen content in the dry weight by the factor of 6.25. Small samples around 20 mg were taken for the ana-

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lyses and hence the digestion with sulphuric acid was carried out in 50 ml Kjeldahl flasks. To obtain the homogenous material the dry carcass was ground in a grinder with rotating blades and then additionally in a porcelain mortar. The dry weight without fat removal was used for the estimations to obtain better homogeneity of the material. Additional determinations of nitrogen in the dry mass after fat extraction showed larger dispersion of results. The caloric value was determined by combusting a sample of tissues in an adiabatic bomb calorimeter (G \circ r c k i, 1965a). In case of very small animals (0 — 6 days of age) the whole carcass was combusted and from the older ones samples of 1 — 1.5 g were used. The ash content was calculated after combustion of the whole body of animals in a muffle oven.

From the described determinations the contents of water, protein, fat and ash were calculated and expressed in relation to the dry mass. The caloric values of dry weight, of ash-free dry weight, as well as the caloric value of biomass and the ash content were calculated from calorimetric determinations. Statistical analysis of the material included calculation of the mean, coefficients of variation (in $^{0}/_{0}$) and correlation. The relationship between the examined body constituents was expressed by means of rectilinear regression equations.

III. RESULTS

The average body weight of voles in the age between 0 and 30 days increased from 1.9 to 16.0 g (Fig. 1, Table 1). Twenty days old animals increased by six times their initial weight at birth, and 30 days old — by eight times. Table 2 shows the maximum, minimum and mean body

Age in days	N	Body weight in g				
		Min.	Max.	Avg. \pm S. D.		
0-1	17	1.38	2.30	1.88 + 0.31		
3	17	2.50	3.38	3.05 ± 0.26		
6	18	3.02	5.39	3.99 ± 0.63		
9	17	4.38	6.98	5.84 ± 0.81		
12	19	5.40	10.80	7.24 ± 1.62		
20	18	10.62	15.34	12.50 ± 1.30		
30	17	11.62	23.42	16.05 ± 3.21		

Table 2.

Body weight of voles during their postnatal development.

weights of voles in all 7 age classes. The most marked increase is observed between 0 and 3 days. The variability of body weight in particular age classes is small and the coefficient of variability is equal to 15.3% on the average.

The data concerning changes of chemical composition of the vole's body are presented with the employment of double-logarithmic scale. The relative contents of water, protein and fat are highly correlated with the body weight of rodents (Figs 2 and 3). In all three cases the coeffi-

cients of correlation are very high (around 0.92 on the average). The regression equations illustrating the relationship between these components are given on the diagrams (Figs 2 & 3).

The water content in the body of voles changes with the age and body weight (Figs 1 & 2, Table 1). The highest content of water was found in the new-born animals (83.7%) and later it decreased to the level of 63.5% in 30 days old animals (Fig. 1). The relative content of water within a particular class of age is rather similar and shows very small variability. The values of standard deviation and coefficient of variability



Fig. 1. Growth curve of common voles within the first 30 days (1) of their postnatal development. Curve of relationship between body water and age (2). Each point shows the mean and one standard deviation (SD) on each side of the mean.

are both low (S.D. ranges from 1.21 to 4.88, C.v. from 1.4 to 7.7%). The percentage of water in the fat-free biomass is slightly higher and remains in the range of 85.8 - 74.0% (Table 3).

The relative content of protein, fat and ash in the body of voles was determined with regard to the dry mass. The percentage of protein apparently decreases during the postnatal development but is highly correlated with the body weight (r = -0.93) (Fig. 2). In the new-born animals the amount of protein is the highest and equal to 65,9%. In three days old it abruptly decreases to 57.5%, in the age between



6 and 20 days it remains on the average level of 52%, and in 30 days old animals it falls to 44.4% (Fig. 4). Within a particular age class only small differences of protein content are visible (*C.v.* ranges from 5.1 to 12.7%), although heavier animals show always lower percentage of proteins. The amount of protein in the fat-free biomass increases from 10.9 to 18.9% during the growth of a vole (Table 3).



Fig. 4. Changes in the gross body constituents of voles (in per cent) during their postnatal development. Each point shows the mean and one standard deviation (SD) on each side of the mean.

The curve of body fatness is just reverse to the protein content: the amount of fat increases in parallel to the biomass (Fig. 3). This correlation (r = 0.82) is, however, not as good as in the two other cases. The mean fat content for the whole material ranges from 17.1 to 39.1% with the lowest values in the youngest animals. In three days old voles the amount of fat abruptly increases to 29.8% and goes up further by a few per cent in 6 days old animals, remaining then on the level around

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33 % until the 12th day. Later the fat content slowly rises reaching 39.1% in 30 days old voles (Fig. 4). When the specimens of the same age are compared the fat content varies to a certain degree and the coefficients of variation are in all cases relatively high ranging from 18.2 to 32.7%.

The ash content in the body of the studied animals is fairly constant. The results obtained both in the calorimetric determinations and after combustion in the oven are very similar. They indicate that the highest ash content is in the new-born animals (around 12%). In other age classes this value remained on relatively constant level ranging from 8.8 to 9.4% (Fig. 4, Table 1). In the fat-free biomass the amount of ash increases progressively with the age of animals from 2.2 to 3.8% (Table 3).

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Composition of vole fat-free fresh weight. Content of water, protein and ash expressed in per cent of fat-free body weight.

Body constituent	Age in days						
	0-1	3	6	9	12	20	30
Fat-free					1918 20		
body weight (g)	1.83	2.85	3.67	5.29	6.54	11.11	13.76
Water (%)	85.8	83.8	81.7	78.6	78.3	76.6	74.0
Protein (⁰ / ₀)	10.9	13.3	14.7	16.4	164	18.4	18.9
Ash (%)	2.2	2.1	2.4	2.8	2.9	3.3	3.8

Table 4.

Energy values of the vole body in different age groups

Age in days		Dry woight	Caloric value in cal/g			
	N	in g	Dry weight ± S.D.	Ash-free dry weight \pm S.D.		
0—1	10	0.31	5,990 + 141	6.822 + 75		
3	10	0.66	$6,228 \pm 323$	6.841 + 351		
6	10	0.99	6.062 ± 173	6.674 ± 196		
9	10	1.68	6.152 ± 334	6.741 ± 326		
12	10	2.12	$6,160 \pm 349$	6.761 ± 331		
20	10	3.99	6.303 ± 203	6.978 ± 166		
30	10	5.86	$6,283 \pm 240$	$6,936 \pm 169$		

The caloric values of dry tissues of voles are comprised within 5990 and 6303 cal/g. The lowest value is observed in the new-born animals. The ash-free dry weight shows slightly higher caloric values equal to 6822 cal/g on the average for the whole material. The dry weight caloric values of the animals from one age class are very similar and the

differences range from 300 to 700 cal/g (C. v. amounting to 1.1 - 5.7%). The energy values of dry mass and ash-free dry weight remain constant during the postnatal development of voles (Table 4).

IV. DISCUSSION

Informations concerning changes of the body constituents in the postnatal development of small rodents are meagre. The percentage composition of the body varies in relation to the age and physiological state of animals and moreover it is subjected to individual variability (M a yn a r d & L o o s l i, 1962).



Fig. 5. Relationship between body water and body protein in the young common voles. The correlation coefficient r = 0.98.

Some authors suggested that the analysis of body constituents should be based on the fat-free biomass since changes in the fat content depend on nutritional conditions (Bailey *et al.*, 1960; Blaxter, 1962; Myrcha & Walkowa, 1968). The amount of fat in the organism exerts a marked influence on the relative content of remaining constituents, particularly water. With respect to the total body composition these two

components are subjected to the greatest variations and when the fat content increases with age the amount of water decreases (Figs 1 & 4). At the same time the protein content undergoes reduction and ash remains on a fairly constant level (Fig. 4). In the fat-free biomass different are not only proportions but even the direction of changes of the examined body constituents. The content of water decreases while the amount of protein and ash rise with age. These changes occur only to a certain age until the animal reaches the so called "chemical maturity". From this moment onwards the composition of the fat-free body remains constant (Bailey *et al.*, 1960). The examined voles show continuous



Fig. 6. The relationship between vole body weight and ratio of protein to water in their bodies.

changes of the fat-free biomass composition during 30 days of observations. It may be supposed that the »chemical maturity« will be achieved later, perhaps together with the sexual maturity.

There exists also a well marked relationship between the water and protein contents (r = 0.98; $Y = 0.2 X^{+1.3030}$) (Fig. 5). With the growth of the animal the content of protein and water decreases, or in other words the relative content of proteins in the biomass is proportional to the hyd-

ration of tissues. B a i l e y *et al.* (1960) proposed a new index of physiological age expressed as the ratio of proteins to water. These authors pointed out the usefulness of the index in the differentiation of animals of the same body weight or the same chronological age. From the results of the present study such index was computed for the voles of various age. The values of this index show a rising trend in parallel to body weight and age of voles. In the period from 0 to 30 days of life the index value increases from 0.120 to 0.330 (Fig. 6). Until the 12th day this index increases abruptly but later the rate of its growth slows down. Similar results for the laboratory mice of the same age were reported by B a il e y *et al.* (1960).

In the organism of a growing vole a clear relationship between the amount of fat and protein also exists. With the increase of body fatness the content of proteins decreases. The coefficient of correlation for this relationship is very high and equal to r = 0.96, and the phenomenon is described by the regression equation: Y = 83.1 - 0.9397 x.

The body composition of a growing vole is generally similar to the analogous data obtained for the laboratory mouse. The content of water found in the laboratory mice by Bailey *et al.* (1960), and by Myrc ha & Walkowa (1968) remains in agreement with the results found in the present study for the vole, both in the relation to the total biomass and to the fat-free biomass. G ó r e c k i (1965b) reported the water content in the new-born *M. arvalis* as equal to 84.9% which is very close to the value obtained in the present study for 0 - 1 day old voles. Laboratory mice show even higher fat content than that of voles (Bailey *et al., l.c.*). The level of proteins in the organism of growing voles rises from 0.20 to 2.60 g, or in other words from 10.9 to 18.9% of the fat-free biomass (Tables 1 & 3). The corresponding data for the laboratory mouse are 0.16 - 2.78 g (Bailey *et al., l.c.*).

The ash content in the laboratory mouse increases with age from 0.03 to 0.58 g (2.3 - 4.2%) of fat-free biomass) (Bailey *et al.*, *l.c.*) hence it is very similar to the values observed in the common vole (Tables 1 & 3). The percentage of ash in the carcass of laboratory mice (Myrcha & Walkowa, 1968), and of voles (Fig. 4) does not change during the postnatal development of these animals. The relative ash content found in the new-born *M. arvalis* (Górecki, 1965b) resembles the value obtained in the present study (12.2 and 11.9\%).

On the other hand the caloric values of the vole's tissues were found to be higher than those reported by M y r c h a & W a l k o w a (1968) for the laboratory mouse. They exceed also caloric values of new-born voles (*Clethrionomys glareolus*, *Microtus arvalis*) and field mice (*Apodemus flavicollis*) as found by G ó r e c k i (1965b). This results probably from

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a higher body fatness of voles reared in the laboratory. The energy values of dry mass and ash-free dry weight for the common vole and laboratory mouse remain on a constant level until 30 days of life, although it seems that the caloric value of the animal body should increase to a certain age (G \circ r e c k i, 1965b). In adult rodents such as *Peromyscus* (G \circ 11 e y, 1962) and *Apodemus* (S a w i c k a - K a p u s t a, 1968) captured in natural habitats the fat content is almost the same as in





young common voles and markedly lower than in 30 days old voles reared in the laboratory.

The present investigations were undertaken to find out the body constituents responsible for the increase of weight of the common vole

during the postnatal development. The quantity of particular body components in a given age is shown in Fig. 7. If the animal during its 20 days of life increases the biomass by six times on the average then the amount of water goes up five times, protein 10 times and fat 27 times. The corresponding figures for 30 days old voles are: water 6.5 times, protein 13 times, fat — as much as 45 times (Table 1, Fig. 7).

It seems that in further studies it would be advisable to analyse the chemical body composition in older age classes in order to see the moment when the organism of the vole reaches the »chemical maturity«.

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Skład chemiczny i wartość kaloryczna ciała polnika zwyczajnego

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ZMIANY SKŁADU CHEMICZNEGO I WARTOŚCI KALORYCZNEJ CIAŁA POLNIKA ZWYCZAJNEGO W ROZWOJU POSTNATALNYM

Streszczenie

U polnika zwyczajnego (*Microtus arvalis*) w wieku od 0 do 30 dni zbadano zmiany składu chemicznego i wartości kalorycznej ciała. Przeanalizowano łącznie 123 zwierzęta, które podzielono na 7 klas wiekowych (0—1, 3, 6, 9, 12, 20, 30 dni) (Tabela 1). Wszystkie oznaczenia składników ciała wykonano w suchej masie. Zawartość tłuszczu w ciele polników określano ekstrahując go eterem w aparacie Soxhlet'a. Białko wyliczano z całkowitej ilości azotu oznaczonego metodą Kjeldahl'a. W piecu do spalań oznaczano zawartość popiołu. Wartość kaloryczną określono spalając próbkę tkanek zwierząt w bombie kalorymetrycznej.

Średni ciężar ciała polników w wieku od 0 do 30 dni wzrastał od 1.9 do 16.0 g (Tabela 1, Ryc. 1). Procentowy udział wody, białka i tłuszczu jest wysoko skorelowany z ciężarem ciała (r = 0.92) (Ryc. 2, 3). Ilość wody w ciele polników zmniejsza się z wiekiem zwierząt. Nowonarodzone polniki posiadają najbardziej uwodnione tkanki (83,7%), potem ilość wody stopniowo maleje, aż w wieku 30 dni osiąga poziom 63,3% (Ryc. 1, 2). Procent białka maleje wyraźnie ze wzrostem. U zwierząt najmłodszych ilość białka jest najwyższa i wynosi 65,9%, u 3-dniowych gwałtownie maleje do 57,5%. W dalszych klasach wiekowych aż do 20 dni utrzymuje się na poziomie około 52,0%, a u 30-dniowych spada do 44,4% (Ryc. 4). Ze wzrostem biomasy i wieku zwierzęcia wzrasta ilość tłuszczu w jego ciele od 17,1 do 39,1% (Ryc. 3, 4). Zawartość popiołu w tkankach badanych zwierząt jest dość stała i mieści się w granicach od 9 do 12%. Wartość kaloryczna suchej tkanki polnika jest zawarta pomiędzy 5990 a 6303 cal/g (Tabela 4). Wskaźnik wieku fizjologicznego wyrażony stosunkiem białka do wody ma tendencję do wzrostu z ciężarem i wiekiem polników od 0,120 do 0,332 (Ryc. 6). W biomasie beztłuszczowej zmieniają się nie tylko proporcje, ale i kierunek zmian między tymi składnikami ciała. Zmniejsza się ilość wody, a ilość białka i popiołu wzrasta z wiekiem zwierzęcia (Tabela 3). Jeśli w ciągu pierwszych 30 dni życia polniki zwiększają swoją biomasę 8-krotnie, to ilość wody wzrośnie 6,5 razy, białka 13 razy, a tłuszczu 45 razy (Ryc. 7).