

Anna DOBROWOLSKA

**Electrophoretic Pattern of Proteins and Glycoproteins in the Blood Serum of Common Voles in Relation to the Season and Physiological Activity \***

[With 1 Table &amp; 1 Fig.]

The differences in the pattern of serum proteins were observed in pregnant females of *Microtus arvalis* (Pallas, 1779). Also some seasonal changes are observed in the level of proteins and glycoproteins. No simple relationship was stated between the age of the voles and the level of protein fractions of the blood serum. There is no relationship between changes in the population density and the level of measured parameters.

## I. INTRODUCTION

Proteins and glycoproteins of the blood serum were investigated in different animal species and in various physiological states, such as growth (Walkowiak *et al.*, 1971; Walkowiak & Skubiszewski, 1970), maturity, pregnancy and lactation in the mare (Walkowiak & Skubiszewski, 1970), ewe (Walkowiak *et al.*, 1971) and in some rodents (Walkowiak *et al.*, 1972).

However, no information is available on the seasonal changes in the level of protein and glycoprotein fractions in the blood serum of voles. The investigation of these changes might supplement our knowledge of physiology of voles, which is important for the explanation of a high reproduction rate and of the regulatory mechanisms governing these populations.

## II. MATERIAL AND METHODS

The blood serum used in the experiments derived from approximately 200 voles captured randomly every month, from May 1971 till June 1972 (except August 1971

\* Praca została wykonana w ramach problemu węzłowego 09.1.7, koordynowanego przez Instytut Ekologii Polskiej Akademii Nauk.

and April 1972 when trapping was not carried out on account of low numbers), in the experimental populations at Łomna near Warsaw. These populations lived on 1 ha plots of alfalfa surrounded by a tight fence. Always 5% of the population numbers were trapped for the investigations.

On the ground of morphological picture of reproductive organs the animals were divided into 7 physiological classes. Among males two classes were distinguished: sexually active or inactive, while among females — five classes. Class 0 — females which as yet not reproduced, classes I, II and III — females in 3 consecutive periods of pregnancy (up to 7, 14 or 21 days, respectively), and class IV — females which have already reproduced and were either lactating or not.

Informations on the age of animals were obtained from the Institute of Ecology PAS, where it was determined on the basis of the weight of eye-lenses (Adamczewska-Andrzejewska, 1973).

The blood was taken from the eye vein after removal of the eye bulb under light ether anaesthesia. The collected serum was pooled within each physiological class.

Plasma proteins were separated by means of paper electrophoresis in the conditions described by Dzułyńska *et al.* (1964). Medinal-veronal buffer pH 9 was used. The runs were carried out in 12-strip chambers on Whatman No. 1 paper during 18 hours at 160 V and  $I=0.1$ . In order to determine proteins slightly less than 0.01 ml of serum was applied to paper strip, while for glycoproteins — 0.05 ml of serum. The electropherograms were stained with amido black to visualize protein fractions, or with Schiff reagents for glycoproteins. Stained strips were evaluated using an ERI-550 Zeiss densitometer.

Since pooled serum was used no statistical analysis of the results was carried out.

### III. RESULTS

After electrophoretic separation of the vole serum 5 fractions of proteins or glycoproteins were obtained (Fig. 1).

The most conspicuous differences between the classes of animals were observed in the level of albumins, alpha-2- and gamma globulins as well as alpha-2- and gamma-glycoproteins. The level of the albumin fraction is higher in males sexually inactive (46.5%) than in active (42.6%). In females the level of albumins decreases during the first week of pregnancy (from 45.5% in class 0 to 38.6% in class I) but it rises again in later periods of pregnancy.

The level of globulin fractions is slightly higher in active than in inactive males, except alpha-1-globulins. The relative values of the content of alpha-2-globulins, both as proteins and glycoproteins increase during pregnancy (class I=12.7%, II=13.9%, III=17.0% for proteins, and I=32.2%, II=35.5%, III=41.1% for glycoproteins), but after parturition they return to the values before pregnancy.

The level of gamma-globulins (both as proteins and glycoproteins) is higher in active males (20.1% of proteins and 15.2% of glycoproteins)

than in non-active (15.0% of proteins and 13.0% of glycoproteins). In females this fraction considerably increases during the first period of pregnancy (by approximately 20% in comparison to group 0), but later it decreases to the value of group 0.

During the summer period of reproduction (from May till September)<sup>1</sup> an increase in the level of albumin fraction was observed (except July). This was accompanied by a decrease in globulin fractions, especially in case of alpha-globulins which are higher by 3—4% in females than in males, while the reverse situation was observed for gamma-globulins (Table 1).

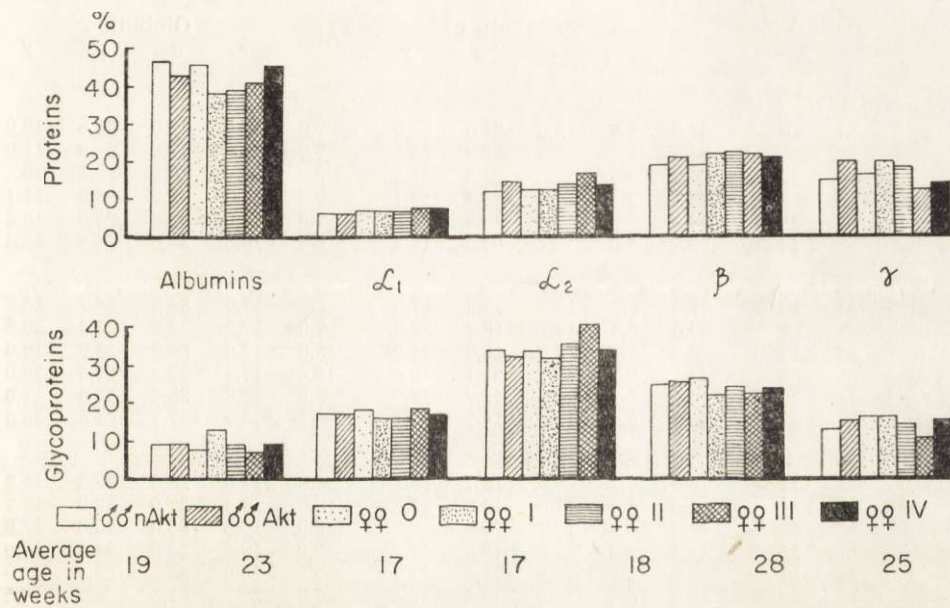


Fig. 1. The mean relative content of protein and glycoprotein fractions in the blood serum of voles (males and females) in various physiological states.

In autumn, at the highest numbers and stabilization of the population (September—November) higher values of albumin content were observed (by 5% on the average) with an abrupt rise of glycoproteins in this fraction in October. In the same period a rise in alpha-1-globulins begins in males, with a concomitant decrease of this fraction in females. The level of gamma-globulins gradually increases in males, while in females it decreases by approximately 4.5% in the period from September to October.

<sup>1</sup> The data on numbers and density of the vole population in Łomna were obtained from Dr. K. Adamczewska-Andrzejewska, Institute of Ecology PAS in Dziekanów.

In winter, during an abrupt decrease in vole numbers (December—February), rather significant alterations in the glycoprotein content of the albumin fraction were observed. The level of alpha-globulin (as proteins) is higher by 1 to 4%, this time in males. Simultaneously a decrease in gamma-globulins (both as proteins and glycoproteins) was ob-

Table 1

The mean relative content of protein and glycoprotein fractions in the blood serum of voles trapped every month during the year.

M — males, F — females; Age means age in weeks; Alb. — albumins.

Month	Sex	Age	Proteins					Glycoproteins				
			Alb.	$\alpha_1$	$\alpha_2$	$\beta$	$\gamma$	Alb.	$\alpha_1$	$\alpha_2$	$\beta$	$\gamma$
Summer 1971												
May	M	49	37.5	7.5	13.0	22.0	20.0	7.0	18.5	30.0	26.5	18.0
	F	32	34.7	7.7	18.0	23.2	16.5	7.0	20.0	37.8	24.3	11.0
June	M	12	44.0	9.0	13.0	21.2	12.3	8.2	16.8	40.8	23.5	10.8
	F	9	42.4	9.7	16.0	19.9	12.1	6.5	18.0	41.5	23.0	11.1
July	M	16	38.0	6.0	16.0	24.5	15.3	7.0	14.0	33.5	27.0	18.5
	F	14	40.7	7.0	15.4	24.6	12.4	6.8	16.6	38.6	24.6	13.4
Autumn 1971												
Sept.	M	16	43.8	6.7	13.2	18.8	17.2	8.2	17.0	34.5	24.7	15.8
	F	12	40.0	6.6	10.7	18.8	23.3	7.0	17.2	32.0	23.3	20.5
Oct.	M	16	48.0	6.0	13.0	18.0	15.0	15.0	16.0	30.0	23.0	16.0
	F	20	43.1	6.7	11.6	20.1	18.8	13.4	17.1	33.4	23.3	14.0
Nov.	M	20	49.2	5.3	11.0	18.7	16.2	5.5	16.8	35.8	25.5	17.0
	F	22	46.7	6.2	11.4	18.7	17.1	6.5	15.8	37.7	24.5	15.5
Winter 1972												
Dec.	M	21	44.5	6.7	10.5	18.0	19.7	18.0	17.5	26.0	23.2	15.3
	F	21	41.0	6.0	11.0	19.7	21.2	16.7	16.3	27.0	22.0	17.7
Jan.	M	22	43.7	6.2	13.7	20.5	15.5	5.5	18.5	38.0	26.0	12.0
	F	25	46.1	5.6	11.0	19.8	17.1	7.8	16.8	33.8	24.7	17.0
Febr.	M	31	48.2	6.7	12.0	19.5	13.5	6.8	20.0	34.2	28.3	11.0
	F	32	48.0	5.5	10.5	18.5	17.5	7.0	20.5	31.5	26.0	14.5
Spring — summer 1972												
March	M	26	44.5	6.0	14.5	20.5	14.5	11.0	19.0	36.0	22.0	13.0
	F	31	38.0	6.0	12.7	24.0	19.2	6.0	16.5	34.0	26.2	17.0
May	M	29	39.0	5.5	13.0	25.5	16.2	15.0	12.5	31.0	25.5	15.5
	F	31	36.0	6.3	14.2	25.2	18.2	15.0	17.3	20.7	22.5	14.7
June	M	34	38.2	7.0	16.3	22.2	16.2	12.2	16.8	34.8	22.8	13.3

served, on the average by 1 to 5%. The relative content of this fraction is higher in females (mean 16.6%) than in males (mean 12.7%).

In spring (from February till May) a decrease in the level of albumins was observed both in males and females (by 6 to 8% on the average) with simultaneous increase of glycoproteins in this fraction. In that period the content of globulin fractions increases but glycoproteins decreases, except gamma-globulins.

## IV. DISCUSSION

The level of glycoproteins in the albumin fraction observed in the present study is on the average lower, while alpha-glycoproteins higher than it was observed in young voles from the *Microtus agrestis* species examined in the postnatal period by Dzułyńska *et al.* (1971). The differences in the level of glycoprotein fractions in these two species may be related to different age, because all the animals trapped for the present investigations were somatically mature, *i.e.*, aged from 9 to 49 weeks.

The most pronounced differences in the pattern of serum proteins were observed in pregnant females. Decrease in the level of albumins observed during pregnancy, similarly to that found in pregnant rabbits (Prusiewicz-Witaszek & Działoszyński, 1967) is probably related to the augmented metabolism in this period (*cf.* Trojan & Wojciechowska, 1967).

Walkowiak & Skubiszewski (1970) observed in pregnant cows, Walkowiak *et al.* (1970) in mice, rabbits, hamsters and guinea pigs, Wójcik & Ewy (1965) in mares, and Prusiewicz-Witaszek and Działoszyński (1967) in rabbits — decrease of the albumin fraction of glycoproteins. In the present study the level of glycoproteins in this fraction considerably increased in the first period of pregnancy and decreased only in the second and third period of pregnancy.

Similarly to the papers cited above also the increase in the content of alpha- and beta-globulin fractions of proteins and alpha-2-glycoproteins was observed. This may be caused by the augmented content of gonadotropins which are known to be glycoproteins (Wójcik & Ewy, 1965).

The gamma-globulin fraction behaves similarly to that in pregnant mares (Wójcik & Ewy, 1965), rabbits (Prusiewicz-Witaszek & Działoszyński, 1967) and hamster (Walkowiak *et al.*, 1972), *i.e.*, its level gradually decreases to the third period of pregnancy.

In the discussed papers changes in the electrophoretic pattern of serum proteins observed during pregnancy persisted, or were even enhanced, during lactation. In the present study no such relationship was observed, probably because pooled blood samples were used in class IV containing both lactating and non-lactating females.

The lower level of albumin and higher level of globulins, and especially of gamma-globulins, in vole males sexually active may be explained by increased metabolism and behavioral activity (Christian, 1963).

Seasonal changes in the albumin level are related to oscillation in the activity of thyroid and basal metabolism in voles as demonstrated by

Rigaudière (1967). Particularly in summer, when general metabolism of the vole increases and thyroid shows maximum activity, lower levels of this fraction are observed.

Higher level of albumin in autumn and winter may testify to the existence of a reserve pool of proteins in the animals prepared for wintering. These animals are known to live longer not reaching sexual maturity in the year of birth (Martinet, 1967).

Lower levels of gamma-globulin fraction, and hence immunoglobulins, might indicate that in this period the resistance of animals to infection is lower than for example in autumn.

Changes in the content of proteins and glycoprotein fractions occurring in winter are interesting: increase of glycoproteins in the albumin fraction (containing among others a carrier for thyroid hormones), as well as decrease in the level of gamma-globulins and glycoproteins observed in males. It should be emphasized that these changes occur in the period without reproduction and at increased mortality of animals (Adamczewska-Andrzejewska, in litt.).

In the examined material no simple relationship was observed between the age of animals and the level of protein fractions of the blood serum. This may be due to the fact that the animals being in the same age but in different seasons differ in respect of morphology and physiology (Martinet, 1967).

Also no relationship was found between changes in the population density and the level of protein fractions, especially of gamma-globulins. According to Christian (1963) increased population density may give reduced level of immuno-globulins on account of increased activity of adrenals. However, at the autumn maximum of the population numbers in Łomna equal to 630 individual for two 1-ha plots rather high levels of gamma-globulins were observed in comparison with summer. Hence the increased density occurring in autumn 1971 cannot be regarded as a stress factor influencing examined parameters.

#### REFERENCES

1. Adamczewska-Andrzejewska K. A., 1973: The lens weight as indicator of age in the wild *Microtus arvalis* population. Bull. Acad. Polon. Sci., Biol., 21, 5: 331—336.
2. Christian I. J., 1963: Mammalian population. [In: »Physiological mammalogy«, Eds. Mayer W. V. & Gelder R. G. van], 1: 189—328. Academic Press, New York and London.
3. Dżułyńska J., Krajewska K. & Gill J., 1964: Serum glycoproteins in some species of non-domesticated mammals. Acta biochim. pol., 11, 2—3: 121—128.

4. Dżułyńska J., Pucek M. & Walkowiak H., 1971: Studies on serum glycoproteins in the voles (*Microtus agrestis*) during the growth period. Bull. Acad. Pol. Sci., Biol. 19, 2: 101—105.
5. Martinet L., 1967: Cycle saisonnière de reproduction du campagnol des champs *Microtus arvalis*. Ann. Biol. Anim. Bioch. Biophys., 7, 3: 245—259.
6. Prusiewicz-Witaszek U. & Działoszyński L., 1967: Wpływ ciąży i laktacji na poziom białek, glikoproteidów i lipoproteidów w surowicy królików. Med. wet., 23: 627—630.
7. Rigaudière N., 1969: Les variations saisonnières du métabolisme de base et de la thyroïde chez les Microtinés. Arch. Sci. Physiol., 23, 2: 215—241.
8. Trojan P. & Wojciechowska B., 1967: Resting metabolism rate during pregnancy and lactation in the European common vole *M. arvalis*. Ekol. pol., 15, 44: 812—817.
9. Walkowiak H., Brylińska J., Krawczyńska M. & Starzyński W., 1972: Studies on serum glycoproteins of some mammal species in various physiological condition. Pol. Archw. wet., 15, 2: 385—395.
10. Walkowiak H., Reklewska J. & Dżułyńska J., 1971: Badania nad glikoproteidami surowicy krwi owiec w okresie wzrostu, ciąży i laktacji. Acta physiol. pol., 22, 2: 273—277.
11. Walkowiak H. & Skubiszewski B., 1970: Serum glycoproteins in cattle during growth and pregnancy. Bull. Acad. Polon. Sci., Biol., 18, 9: 533—538.
12. Wójcik K. & Ewy Z., 1965: Studies on the serum glycoproteins level in mares early pregnancy. Bull. Acad. Polon. Sci., Biol., 13, 8: 485—487.

Accepted, March 8, 1975.

Department of Animal Physiology,  
University of Warsaw,  
Zwirki i Wigury 93,  
02-089 Warszawa, Poland.

Anna DOBROWOLSKA

POZIOM ELEKTROFORETYCZNYCH FRAKCJI BIAŁKOWYCH  
I GLIKOPROTEIDOWYCH SUROWICY KRWI NORNİKÓW W ZALEŻNOŚCI  
OD SEZONU I AKTYWNOŚCI FIZJOLOGICZNEJ

Streszczenie

Badano procentową zawartość elektroforetycznych frakcji białkowych i glikoproteidowych surowicy krwi norników odławianych przez 1 rok z populacji izolowanych założonych przez Instytut Ekologii PAN na jednohektarowych polach lucerny w Łomnej k/Warszawy.

Stwierdzono, że największym wahanom sezonowym podlega frakcja albuminowa, alfa<sub>2</sub>- i gamma-globulinowa białek oraz albuminowa, alfa<sub>2</sub>- i gamma-globulinowa glikoproteidów. Największe zmiany poziomu tych frakcji obserwuje się u samic ciężarnych.

Zmiany sezonowe poziomu badanych frakcji są spowodowane różnym natężeniem metabolizmu w różnych sezonach, jak i tym, że osobniki będące w tym samym wieku, ale z różnych pór roku nie odpowiadają sobie nawzajem morfologicznie i fizjologicznie.