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Activity of Blood Serum Enzymes in Seasonal Generations of the Bank Vole

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Wołk E. & Józefczak E., 1980: Activity of blood serum enzymes in seasonal generations of the bank vole. Acta theriol., 25, 30: 377—384 [With 2 Table and 2 Figs.]

Studies were made on the activity of the following blood serum enzymes: alkaline phosphatase (*AP*) and phosphocreatine kinase (*CPK*) in 84 voles either 30 or 60 days old, obtained from spring and autumn seasonal generations. The body weight of 30- and 60-day old voles born in spring was greater than that of rodents of the same age born in autumn ($P < .001$, $.005 < P < .01$ respectively). A negative correlation was found, with coefficient $r = -0.592$ ($P < .001$) between body weight and *AP* activity. *AP* activity decreases with age in voles and differences between 30- and 60-day old rodents are significant: in spring $P < .001$, in autumn $.005 < P < .01$. The distinctly higher level of *AP* activity in voles 30 days old, from the autumn generation (1292.9±512.6 IU) than in those from the spring (790.6±192.8 IU) thus shows that growth and ossification of the skeleton, processes connected with *AP* activity, take place differently in extreme seasonal generations. The rate of these processes is more rapid in the spring generations, and consequently there is a divergence between absolute and physiological age of voles born at different seasons of the year. The fluctuations observed in *CPK* activity, on the other hand, are connected more with a change in the activity of these animals.

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1. INTRODUCTION

It was found that the rate of growth and development varies in young bank voles, *Clethrionomys glareolus* (Schreber, 1780) depending on the season of birth (Schwarz *et al.*, 1964; Zejda, 1971). Voles from the spring generations are distinguished by quicker rate of growth, quicker attainment of sexual maturity and higher fecundity than voles of the autumn generation (Newson, 1963; Kubik, 1965; Claude, 1970; Crawley, 1970), and also a higher rate of metabolism during postnatal development (Gębczyński, 1975). As early as the first day to life individuals born in spring have a different chemical composition of the body from those born in autumn, and the rate of attainment of chemical maturity is different in these two generations (Fedyk, 1974). All the foregoing points to difference between absolute and

physiological age of voles from spring and autumn generations, and to the more rapid ageing of voles born in spring.

A comparison is given in this paper of the activity of two blood serum enzymes, alkaline phosphatase (*AP*) and phosphocreatine kinase (*CPK*) in voles of the same age, but originating from different generations, based on the assumption that differences in rate of growth and development of mammals should be reflected in the level of enzymatic processes in the tissues.

2. MATERIAL AND METHODS

A total of 84 voles, either 30 or 60 days old, were used for the studies. The spring generation in 1977 consisted of young voles produced by gestating females caught in the Białowieża National Park in April 1977. Further autumn and spring generations were obtained each time from paired adult animals caught in BNP in August 1977, which in this way formed the parent pool. They were kept in cages, provided with water ad lib. and fed on oats, with carrot or beetroot.

In 1977 and 1978, twice a year, *i.e.*, in May and September or January, 30-day old voles were decapitated in 1977, and 30- and 60-day old in 1978, collecting the flowing blood in test-tubes and centrifuging the coagulated blood for serum. The serum was next frozen at a temperature of −13°C and kept in this state for further examination. Sex and body weight (after loss of blood) were noted for the animals examined.

AP activity was determined in 84 voles, using sets of Eskalab tablets and an Eskalab spectrophotometer. An Eskalab tablet contains the reagents required to determine alkaline phosphatase activity in serum by means of the modified Bessey *et al.* method (1946). *CPK* activity was determined in 61 voles using Eskalab tablets and an Eskalab spectrophotometer, and Oliver's method (1955) as modified by Rosalka (1967) and Nielson & Ludwigsen (1963). *CPK* determination is based on the Wartburg optical test with auxiliary and indicator reaction (Richterich, 1971).

The significance of differences between mean values of enzymatic activity was checked by test *t* for two independent groups.

3. RESULTS

1. Alkaline Phosphatase

No differeces were found between *AP* activity in males an females. *AP* activity in 30-day old voles from the springs of 1977 and 1978 and from the autumn of both years does not differ statistically. Data for the respective seasons for both years were therefore considered jointly (Table 1, 2, Fig. 1).

The differences between mean body weight of these mammals in corresponding age groups for the two years were significant ($P < .001$). The body weight of 30-day old voles from spring generations was greater than that of voles of the same age from autumn generations, in both

the study years ($P < .001$). 60-day old voles born in autumn were also lighter in weight than voles born in spring, although this difference is smaller ($.005 < P < .01$, Table 1). A negative correlation was shown to occur, with coefficient $r = -0.592$ ($P < .001$), between the body weight of voles and *AP* activity (Fig. 2).

AP activity in 30-day old voles of the autumn generation (1292.9 IU) was higher than in the spring generation (790.6 IU), ($P < .001$, Fig. 1).

Table 1

Activity of blood serum enzymes (in IU and body weight of bank voles of different generations.

Age, days	Generation	Body wt., g $\bar{x}\pm$S.D.	Alkaline phosphatase (*AP*) n	$\bar{x}\pm$S.D.	Phosphocreatine kinase (*CPK*) n	$\bar{x}\pm$S.D.
30	Spring '77	15.7±1.5	24	762.5±178.9	8	2678.± 684.4
30	Spring '78	10.1±1.5	8	875.0±208.1	5	4188.0±1936.5
30	Spring '77+'78	—	32	790.6±192.8	13	3259.2±1506.6
60	Spring '78	15.0±1.5	10	513.5±219.1	9	1455.5± 878.3
30	Autumn '77	13.0±2.5	10	1184.0±376.6	12	4732.5±1702.2
30	Autumn '78	7.2±1.2	18	1353.3±565.4	13	2225.0±1320.0
30	Autumn '77+'78	—	28	1292.9±512.6		—
60	Autumn '78	12.6±1.9	14	794.3±441.8	14	2314.3±1815.1

Table 2

Comparison of significance of differences in the activity of blood serum enzymes in bank voles of different age groups and generations.

	Age, days	Year	Generation	*P*	N
AP	30	'77—'78	spring	$P > .05$	32
	30	'77—'78	autumn	$P > .05$	28
	30	'77+'78	spring—autumn	$P < .001$	60
	60	'78	spring—autumn	$P > .05$	24
	30—60	'77+'78	spring	$P < .001$	42
	30—60	'77+'78	autumn	$.002 < P < .005$	42
CPK	30	'77—'78	spring	$P > .05$	16
	30	'77—'78	autumn	$P < .001$	25
	30	'77+'78	spring—autumn '77	$.005 < P < .01$	25
	30	'77+'78	spring—autumn '78	$P > .05$	26
	60	'78	spring—autumn	$P > .05$	23
	30—60	'77+'78	spring	$.005 < P < .01$	22
	30—60	'77—'78	autumn '77	$.002 < P < .005$	26
	30—60	'78	autumn	$P > .05$	27

Differences between *AP* activity in 30- and 60-day old voles are statistically significant (in spring $P < .001$, in autumn $.002 < P < .005$). The activity of this enzyme thus decreases with increasing age of the voles, whereas 60-day old voles from spring and autumn do not differ in respect of *AP* activity.

2. Phosphocreatine Kinase

No differences in *CPK* activity vere found between males and females. *CPK* activity in 30-day old voles of spring generations in both study years did not differ statistically and these data have been considered jointly. The activity of this enzyme differs, however, in autumn generations ($P < .001$) and differing tendencies occur in the two years. In spring voles *CPK* activity was 3259.2 IU, and was lower than in voles born in the autumn of 1977 (4732.5 IU, $.005 < P < .01$), while it was greater than *CPK* activity in the autumn of 1978 (2225.0 IU), although this difference was not statistically significant (Fig. 1, Tables

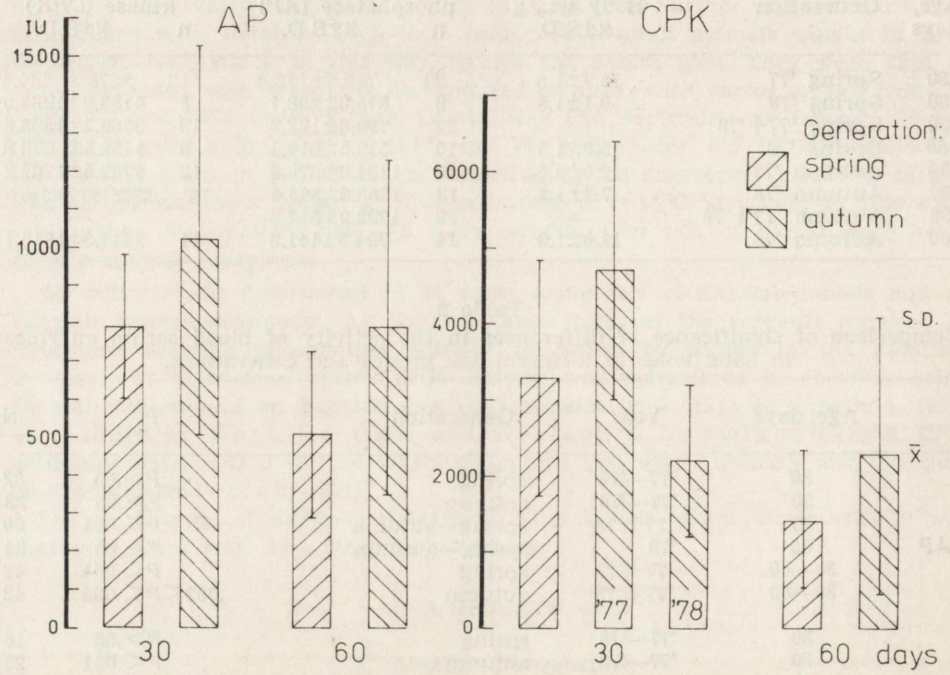

Fig. 1. Activity of blood serum enzymes in bank voles of different generations.

1, 2). There is no correlation between the body weight of these rodents and *CPK* activity, neither are there any differences in the activity of this enzyme between 60-day old animals from spring and autumn.

4. DISCUSSION

Alkaline phosphatase is a non-specific enzyme primarily playing a part in transport of metabolites through the cell membrane and also

in forming protein fibres. The role of *AP* in skeleton ossification processes is well known. It catalyzes the hydrolysis reaction of orthophosphate monoesters. The serum of adult humans contains chiefly liver isoenzyme and trace amounts of bone isoenzyme. Bone *AP* occurs in large amounts in the serum of children. Variations in *AP* activity have been shown to occur in various organs in many small mammals: in the kidneys, skin and brown fat, depending on the animals' age and season during the yearly cycle, or on ambient temperature (Hyvärinen, 1968, 1969a; Pasanen, 1969, 1970; Hyvärinen *et al.*, 1971; Haarakangas, 1972).

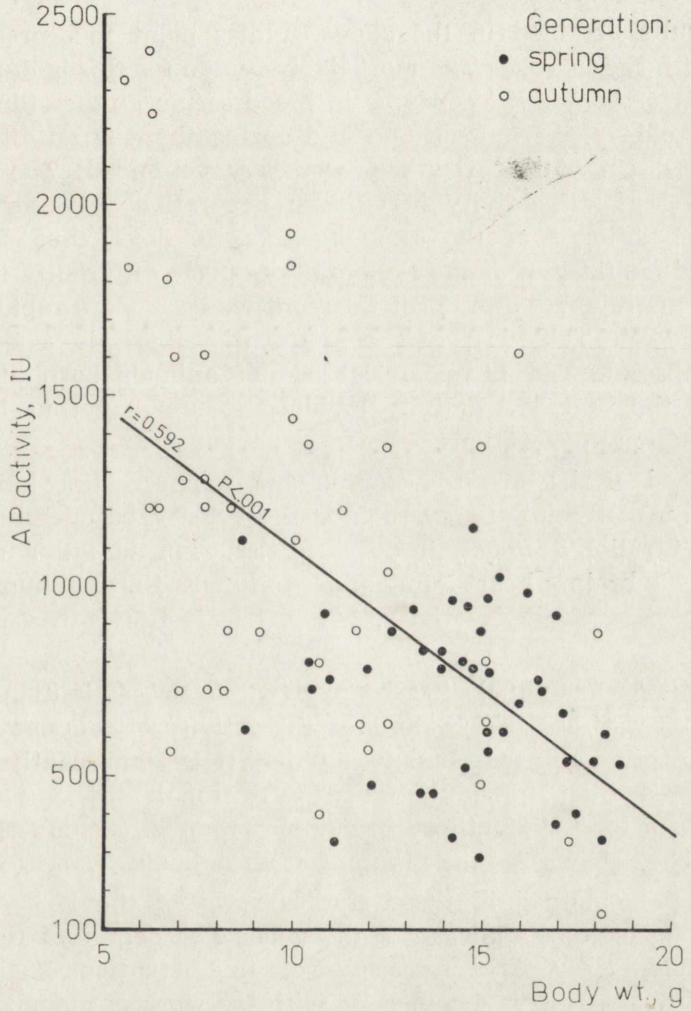

Fig. 2. Correlation between body weight and alkaline phosphatase activity (*AP*) in the bank vole.

In the bank vole Hyvärinen (1968) showed that increase in AP activity in kidneys may reflect variations in metabolism connected with the organism's growth, and consequently with the considerable secretion of somatotropin and protein synthesis. The activity of this enzyme examined in the limb bones if the bank vole may be treated as an indicator of this mammal's growth. In general this author found differences in *AP* activity depending on the season and the rodents' age (Hyvärinen, 1969b). In young growing reindeer calves (*Rangifer tarandus*) there is distinct increase in *AP* ativity in the blood serum, particularly in summer, in comparison with adult individuals (Hyvärinen *et al.*, 1977).

The results obtained in the above studies point to decrease in *AP* activity with age in voles. The most likely reason for this is the diminishing amount of bone *AP* isoenzyme in blood serum as the animals grow. In 60-day old voles from autumn and spring there is no difference in *AP* activity, since the growth processes have ended. The markedly higher level of *AP* activity in autumn generation voles as compared with spring animals of the same age, *i.e.* 30 days, thus shows that growth and ossification of the skeleton take place differently in extreme seasonal generations. The rate of these processes is more rapid in spring generations (cf. Table 1), and consequently there is a discrepancy between absolute and physiological age of animals born at different seasons of the year. *AP* activity in blood serum may thus be used to describe seasonal generations.

The second of the enzymes examined, phosphocreatine kinase, is an enzyme connected with energy balance and is localized in cell cytoplasm. *CPK* concentration decreases in different tissues in the following sequence: muscles > brain> heart > intestines > lungs. Normal human serum contains a trace of *CPK* activity, but physical effort raises its activity in blood.

CPK activity in animals has been given far less attention than *AP*. Hyvärinen *et al.* (1976) examined the activity of this enzyme in the serum of captive reindeer and found that it is significantly higher in young calves than in adult females. Seasonal differences, namely higher values for *CPK* activity in summer than in autumn and winter, have also been shown for the group of adult animals.

The results obtained in these studies revealed the lack of relation between *CPK* activity and age of the voles. The degree of the animal's activity undoubtedly plays a decisive role in fluctuations in the activity level of *CPK* — an enzyme connected with the work of mammal muscles.

Comparison of absolute values for activity of the two enzymes examined in the bank vole with data obtained by different authors is

useless, in view of the decisive effect exerted by study methods on the data obtained. The very high level of these indexes in the bank vole in comparison with other mammals or man generally merits attention. The small body dimentions of these rodents undoubtedly influenced this. *AP* activity in the serum of adult laboratory rats, measured by the same method as that in our studies does not exceed 200 IU (Józefczak, in litt.).

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AKTYWNOŚĆ ENZYMÓW SUROWICY KRWI U GENERACJI SEZONOWYCH NORNICY RUDEJ

Zbadano ciężar ciała oraz aktywność enzymów surowicy krwi: fosfatazy alkalicznej (*AP*) i kinazy fosfokreatynowej (*CPK*) u 84 nornic, *Clethrionomys glareolus*, w wieku 30 i 60 dni, pochodzących z wiosennych i jesiennych generacji sezonowych.

Ciężar ciała 30- i 60-dniowych nornic z wiosny był wyższy, niż ciężar ciała gryzoni w tym samym wieku, urodzonych jesienią ($P < .001$, $.005 < P < .01$ odpowiednio, Tabela 1). Stwierdzono ujemną korelację o współczynniku $r = -0.592$ ($P < .001$) między ciężarem ciała i aktywnością *AP* u badanych nornic (Ryc. 2) oraz brak takiej korelacji między ciężarem ciała a aktywnością *CPK*.

Aktywność *AP* zmniejsza się z wiekiem nornic, różnice między 30- i 60-dniowymi gryzoniami są istotne: wiosną $P < .001$, jesienią $.005 < P < .01$. Wyraźnie wyższy poziom aktywności *AP* u nornic w tym samym wieku 30 dni z generacji jesiennej (1292.9 jM) niż wiosennej (790.6 jM) wskazuje więc, że wzrost i kostnienie szkieletu, procesy związane z aktywnością *AP*, przebiegają różnie w skrajnych generacjach sezonowych (Tabele 1 i 2, Ryc. 1). Tempo tych procesów jest szybsze w generacjach wiosennych, w związku z czym istnieje rozbieżność między wiekiem bezwzględnym a fizjologicznym nornic, pochodzących z różnych generacji sezonowych. Aktywność AP surowicy krwi może więc służyć do charakterystyki generacji sezonowych.

W przypadku *CPK*, enzymu związanego z gospodarką energetyczną, nie stwierdzono takich współzależności (Tabele 1 i 2, Ryc. 1). Obserwowane wahania w aktywności *CPK* są raczej związane ze zmianą ruchliwości nornic.