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ORGAN DISTRIBUTION OF SOME CLINICALLY IMPORTANT ENZYMES IN MINK

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JUOKSLAHTI, T., P. LINDBERG and J. TYÖPPÖNEN: *Organ distribution of some clinically important enzymes in mink.* Acta vet. scand. 1980, *21*, 347—353. — The activity and relative distribution of eight clinically important enzymes were measured in nine different organs in 10 healthy minks. Of the enzymes studied, OCT, ASAT and ALAT had higher absolute activities when compared to many other animals. This is believed to be adaptation to a high protein diet. OCT shows absolute liver specificity, and even ALAT is relatively liver specific in mink. SDH is found in relatively high concentrations in the liver as well as in the kidney. The organ distribution of the other enzymes investigated in mink — AP, CK, γ-GT and LD — is much the same as in many other animal species. Their clinical significance in serum is therefore the same.

enzyme activities; mink organs.

The determination of cellular enzyme activity in serum is a common tool for the diagnosis of various diseases. Elevated serum levels of most cellular enzymes are a sign of cell damage or altered membrane permeability. It has been demonstrated that those enzymes most active in a given tissue appear to leak most from the tissue, although some factors may alter this phenomenon. Among these factors influencing the serum enzyme level is the intracellular distribution and elimination rate from the circulatory system of these enzymes (*Freedland & Kramer* 1970).

The absolute enzyme content and even the relative distribution of enzymes in different tissues are not necessarily the same in different species of animals. The purpose of this study was to determine the absolute and relative enzyme levels of some organs in healthy mink (Mustela vison). A knowledge of the organ content of enzymes is a prerequisite for evaluating serum enzyme pattern in future diagnostic work on various dietary and disease conditions in mink.

MATERIAL AND METHODS

Ten male minks were obtained from the Experimental Ranch of the Finnish Fur Breeders' Association where they were reared on normal ranch diet consisting approximately of 45 % crude protein, 13 % ash, 19 % crude fat and 23 % crude carbohydrates on a dry matter basis. The animals were of the age of seven months and in normal healthy condition. The minks were killed by cervical dislocation, after which tissue samples of liver, kidney, pancreas, spleen, heart muscle, skeletal muscle, stomach, duodenum and jejunum were removed and immediately frozen in carbon acid ice.

For analysis 1 g portions of each tissue were homogenized in 9 ml of cold medium (water or buffer solution) in a Potter-Elvehjelm apparatus. Water was used as the medium for determination of mitochondrial enzyme activity and for osmotic destruction of mitochondrial membranes. The homogenate was then centrifuged for 10 min at 750 $\times$ g.

The determination of enzyme activity in the supernatant was performed on the day the homogenization was carried out, or the supernatant was deep frozen (—20°C) until analysis.

Ornithine carbamoyltransferase (OCT; EC 2.1.3.3.) was measured according to the manual method of *Ohshita et al.* (1976), except that the incubation time at 37°C was increased from 20 min to 45 min to obtain better sensitivity at low enzyme concentrations.

Sorbitol dehydrogenase (SDH; EC 1.1.1.14) was determined with a modification of the manual method of *Gerlach & Hiby* (1974), adapted to a Gilford System 3500 Computer Directed Analyzer (Gilford Instrument Company, Oberlin, Ohio, USA). Analyses were carried out with a program GOT optimized at 37°C with final reagent concentrations 0.12 M, pH 7.4 Tris-buffer, 0.18 mM NADH and 0.102 M D (—) fructose.

Aspartate aminotransferase (ASAT or GOT; EC 2.6.1.1.), alanine aminotransferase (ALAT or GPT; EC 2.6.1.2.), alkaline phosphatase (AP; EC 3.1.3.1), creatine kinase (CK; EC 2.7.3.2), γ-glutamyltransferase (γ-GT; EC 2.3.2.2.) and lactate dehydrogenase (LD; EC 1.1.1.27) in tissue homogenates were determinated with a Gilford 3500 Analyzer at 37°C with Boehringer Mannheim optimized Auto-Tests.

RESULTS AND DISCUSSION

The results of the analysis of absolute enzyme activity in the organs are presented in Table 1 and the results of the relative distribution in Table 2.

Highest concentrations of OCT were in liver, the absolute value being 136.2 μkat/g · 10^{-2} while activity in other organs was negligible. Liver values are higher than those reported for humans (*Cathelineau et al.* 1972) and pigs (*Wretlind et al.* 1959). Compared to humans the dogs have much lower relative organ activity in the intestines (*Reichard* 1960), as was also the case with mink.

Table 1. Absolute enzyme activities in mink organs, μkat/g . 10^{-2} fresh weight (mean ± s, n = 10).

Organ	OCT	SDH	AP	ASAT	ALAT	CK	γ-GT	LD
Liver	136.2	29.3	4.7	325.1	300.6	51.2	0	486.4
	11.2	6.2	2.5	83.7	124.9	28.0		550.1
Kidney	0	19.7	81.5	177.0	31.3	25.8	139.0	22.3
		5.0	48.3	90.2	17.5	19.8	26.5	13.2
Pancreas	0	4.2	3.5	43.2	29.7[b]	4.5[c]	104.2	9.0[a]
		1.7	1.5	9.5	15.0	2.0	20.3	13.3
Spleen	na	0.3	5.0[a]	20.7	4.7	19.5	na	76.5
		0.2	3.2	4.3	1.7	8.0		52.0
Heart muscle	0	0.8	0.8[a]	381.3[a]	69.8[a]	535.4[a]	0.3[b]	415.1[a]
		0.2	0.3	57.8	12.8	213.4	0.1	133.4
Skeletal muscle	0	0.2	0.8	281.1	28.3	634.3	0.5	728.0
		0.1	0.3	49.3	7.8	166.0	0.1	195.7
Stomach	na	0.7	3.3	83.7	33.0	477.9	na	92.5
		0.3	4.5	23.0	6.2	192.5		50.8
Duodenum	0.7	0.2	30.2	36.3	2.8	83.4	10.7	102.0
	0.2	0.1	15.2	6.8	0.8	79.2	2.8	55.5
Jejunum	1.0	0.2	33.7	41.3	4.0	51.0	11.0	91.2
	0.3	0.1	21.8	7.2	1.8	23.3	2.0	38.0

na = Not analysed.
[a] n = 9.
[b] n = 8.
[c] n = 5.

Table 2. Relative distribution of enzyme avtivities in mink organs. Highest mean value per enzyme = 100.

Organ	OCT	SDH	AP	ASAT	ALAT	CK	γ-GT	LD
Liver	100	100	5.8	85.3	100	8.1	0	66.8
Kidney	0	67.2	100	46.4	10.4	4.1	100	3.1
Pancreas	0	14.3	4.3	11.3	9.9	0.7	75.0	1.2
Spleen	na	1.0	6.1	5.4	1.6	3.1	na	10.5
Heart muscle	0	2.7	1.0	100	23.2	84.4	0.2	57.0
Skeletal muscle	0	0.7	1.0	73.7	9.4	100	0.4	100
Stomach	na	2.4	4.1	22.0	11.0	75.3	na	12.7
Duodenum	0.5	0.7	37.1	9.5	0.9	13.1	7.7	14.0
Jejunum	0.7	0.7	41.4	10.8	1.3	8.0	7.9	12.5

na = Not analysed.

The highest concentrations of SDH were found in liver, 29.3 $\mu kat/g \cdot 10^{-2}$, and in kidney, 19.7 $\mu kat/g \cdot 10^{-2}$, respectively. The concentrations of SDH in other organs were low. The absolute value in liver was higher than that reported for humans (*Gerlach & Hiby* 1974), horses (*Gerber* 1969) and cows (*Frahm et al.* 1978), and lower than that for piglets (*Flückiger* 1977). The relative value in kidney was considerably higher in minks than that reported for horses (*Gerber*), humans (*Gerlach & Hiby*), piglets (*Lindberg et al.* 1962, *Flückiger*) and humans (*Asada & Galambos* 1963), thus the liver specificity of SDH in mink is not as high as in other compared species.

AP was encountered in the highest concentrations in kidney at a value of 81.5 $\mu kat/g \cdot 10^{-2}$, while the intestinal concentration was about 40 % of this figure. Concentrations in the other organs ranged between 1 and 6 % of the kidney concentration. The relative organ distribution corresponds well with that for piglets aside from the higher relative intestinal activity in piglets. Absolute activity in piglet organs is considerably lower (*Flückiger*). Humans also have higher absolute and relative activities in intestines when compared to mink (*Schmidt & Schmidt* 1967).

ASAT activity was highest in heart muscle, with an absolute value of 381.3 $\mu kat/g \cdot 10^{-2}$. Skeletal muscle and liver had nearly the same values, but the activity in other investigated organs was lower. Relative organ distribution corresponds well with those reported for man and other animal species (*Cornelius et al.* 1959, *Wretlind et al., Asada & Galambos, Schmidt & Schmidt, Flückiger*), although the absolute values in mink are considerably higher than those reported for piglets (*Flückiger*) and man (*Schmidt & Schmidt*).

ALAT was found to be liver specific in mink, the absolute values being 300.6 μkat/g · 10^{-2}; the relative values for other investigated organs were between 0.9 and 23.2 % when compared to liver. The relative organ distribution is much the same as in dogs (*Cornelius et al.*), humans (*Schmidt & Schmidt*) and cats (*Cornelius & Kaneko* 1960). The absolute value in mink liver is considerably higher than in piglets (*Flückiger*) and man (*Schmidt & Schmidt*).

The highest concentration of CK was in skeletal muscle and heart muscle, where the absolute values were 634.3 and 535.4 μkat/g · 10^{-2}, respectively. The stomach also had high activity. The relative activity of the other organs lay between 0.7 and 13.1 % of the skeletal muscle level. The relative organ distribution is much the same as in man (*Schmidt & Schmidt*) and piglets (*Flückiger*).

The highest activity of γ-GT was recorded in kidney with an absolute value of 139.0 μkat/g · 10^{-2}. Pancreas had 75 % of this activity, while activity in the other investigated organs was much lower. Data obtained for mink corresponds well with those reported for cows (*Rico et al.* 1977 a), pigs (*Rico et al.* 1977 b), ewes (*Braun et al.* 1978), horses (*Rico et al.* 1977 c) and dogs and rats (*Szasz* 1974).

LD activity was highest in skeletal muscle. The absolute value in this tissue was 728.0 μkat/g · 10^{-2}, compared to 66.8 % and 57.0 % of this value for the relative activity in liver and heart muscle and much lower activity in other organs. LD activity in mink organs are between the coresponding activity in piglet organs (*Flückiger*) and in humans (*Schmidt & Schmidt*).

The absolute activities of the ASAT and ALAT transaminases and the urea cyclus enzyme OCT in mink organs were found to be on a considerably higher level than for humans (*Cathelineau et al.* 1972) and pigs (*Wretlind et al.*). An increasing protein level in the diet is known to elevate the levels of these enzymes in organs; *Schimke* (1962) has shown a threefold increase in OCT activity in rat liver after feeding a diet containing 60 % casein, as opposed to a diet containing 15 % casein, for two weeks. Similarly transaminase levels have been demonstrated to elevate considerably with an increasing dietary level of protein in rats (*Knox & Greengard* 1965). The high activities of ASAT, ALAT and OCT in mink liver is probably due to metabolic adaptation to the high protein diet.

Brain tissue has high activity of ASAT and bone tissue has high activity of AP in man (*Schmidt & Schmidt*). These organs were not investigated in this study; their significance, therefore, in the serum pattern of enzymes in mink remains to be investigated.

The activity and great liver specificity of OCT and ALAT seems to be promising in using these enzymes to diagnose liver conditions. The clinical significance of the other investigated enzymes is probably similar for other animals.

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SAMMANFATTNING

Några kliniskt viktiga enzymers fördelning på olika organ hos mink.

Aktiviteten och den relativa fördelningen av åtta kliniskt viktiga enzymer mättes i nio olika organ hos tio friska minkar. Av de undersökta enzymerna hade OCT, ASAT och ALAT högre absolut aktivitet än hos andra djurarter. Det förmodas vara ett tecken på en adaptation till en hög proteinhalt i fodret. OCT är leverspecifik; även ALAT är relativt leverspecifik. Den höga halten av SDH i njurarna innebär att detta enzym inte i samma grad är leverspecifikt. Organdistributionen av övriga enzymer kontrollerade i denna undersökning — AP, CK, γ-GT och LD — är ungefär densamma som hos många andra djurarter. Variation i serumvärdena av dessa enzymer har alltså ungefär samma kliniska betydelse hos mink som hos dessa.

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