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GLYCOGEN IN THE MUSCLES OF RATS POISONED BY METAL IONS

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Glycogen was determined in the skeletal muscles of rat after exposure to several salts of mercury, cadmium, zinc, copper, molybdenum, lead, cobalt and manganese. Treatment with these metals depleted muscle glycogen except in the case of copper and zinc. The causes and significance of the changes involved are discussed.

Nutritional muscular dystrophy, a degenerative disease of striated muscles, has been reported in a wide range of animal species. Trace elements, amongst several other factors, are also known to affect the structure and function of muscles. Excess of manganese causes muscular degeneration (1, 2). Cadmium and zinc have also been reported to alter muscular function (3). Since metals are known to disturb carbohydrate metabolism (4) their involvement with glycogen can also be appraised. Several metals deplete hepatic glycogen. However, their effects on muscle glycogen have still to be investigated. It is known that muscles can form glycogen only from glucose whereas liver can synthesize it from lactic acid, pyruvic acid, glycerol, certain amino acids as well as from glucose. The mechanism of metallic interference might therefore be different for the liver and muscles. Considering this aspect, glycogen was determined in the muscles of rats fed on several environmentally significant salts of mercury, lead, cadmium, zinc, copper, molybdenum, chromium, cobalt and manganese.

MATERIALS AND METHODS

One hundred rats (*Rattus rattus* albino) weighing on average 100 ± 10g and about 90 days old, were selected at random from laboratory stock and placed into ten groups each containing ten rats. The rats were housed individually in suitable cages, and fed on laboratory food (Hindustan Lever Ltd., Bombay). They received water *ad libitum* and were maintained under standard laboratory conditions as described earlier (5). After

Table 1.

Experimental design for treatment (30 days) with heavy metal ions

Group	Metallic salt (in water solution)	Dosage (mg/kg body wt)	Means
A	Control	–	Saline solution
B	Mercuric nitrate	5	Gavage
C	Lead nitrate	5	-||-
D	Cadmiun sulphate	500	-||-
E	Ammonium molybdate	1.000	-||-
F	Copper sulphate	100	-||-
G	Zinc acetate	5.000	-||-
H	Potassium chromate	500	-||-
I	Manganous chloride	250	-||-
J	Cobalt acetate	50	-||-

having acclimatized with laboratory conditions all the animals in a group were administered sub-lethal doses of each metal every day for 30 days by gavage. Details of the present experimental design are shown in Table 1. After scheduled exposure, rats were fasted overnight and then killed by decapitation. Skeletal muscles from hind limbs were quickly removed and processed for determination of glycogen (6). Student's t-test was employed to calculate significant levels between control and experimental values (7).

RESULTS AND DISCUSSION

Exposure to these metal salts, except copper and zinc, depleted glycogen in skeletal muscles. The lowest values were obtained for molybdenum followed by chromium whereas zinc was found to be a better stimulant than copper. However, results were found significant for mercury, lead and cadmium. They are presented in Table 2.

Major biochemical processes, found to be involved in the metabolism of carbohydrates, can be distinguished as catabolic and biosynthetic pathways that embrace the processes such as glycogenolysis, glycogenesis, gluconeogenesis and alternative routes of glucose utilization. A study of hepatic glycogen has also revealed that cadmium, mercury, cobalt, molybdenum, manganese and chromium deplete glycogen whereas copper and zinc promote gluconeogenesis (8). However, literature shows that muscles have no gluconeogenesis.

Accelerated glycogenolysis occurs due to the activation of glycogen phosphorylase and also the action of hydrolytic enzymes. Depression in glycogen transferase limits glycogen storage. Moreover, skeletal muscles alternate between prolonged rest and attainment of maximal activity and require adenosine triphosphate for energy which is generated from glycolysis using store muscle glycogen as well as blood glucose. A

Table 2.

Glycogen content in the muscles of rats poisoned by metal ions

Treatment	Glycogen (g/100 g wet muscle)	Significance level	Alterations %
Control	1.68 ± 0.085	–	–
Mercury	1.42 ± 0.092	2.320*	−15.5
Lead	1.46 ± 0.088	2.010*	−13.1
Cadmium	1.38 ± 0.104	2.497*	−17.9
Molybdenum	0.64 ± 0.042	12.264**	−61.9
Copper	2.72 ± 0.126	7.650**	+61.9
Zinc	2.84 ± 0.132	8.260**	+69.0
Chromium	0.92 ± 0.060	8.166**	−45.2
Manganese	0.96 ± 0.058	7.822**	−42.9
Cobalt	1.02 ± 0.065	6.896**	−39.3

Values expressed as mean ± SD (5 observations) two sets.
+ = % stimulation and − = % inhibition
* Non-significant, ** Significant at $P < 0.001$.

decreased glycogen level in muscles may be due to their maximal activity and hyperglycolysis (9, 10). Available literature reveals that lactic acid, a product of glycolysis in muscles, reaches the liver by circulation and converts into glycogen. Blood glucose supplied by the liver to muscles is converted into glycogen as stored carbohydrates. An increase in the glycogen content of muscles may also be due to the sluggish behaviour of rats (11, 12).

Considering all these mechanisms, it could be assumed that muscle glycogen may have declined in response to the fall in the activity of phosphorylase, through which mechanism formation of glucose-6-phosphate is reduced. Diminished availability of hexose-phosphate thus slows down the conversion of glucose into glycogen. However, this effect might be secondary to adrenal stimulation originating from primary sympathetic activation. It can be concluded that glycogen disorders may contribute to muscular dystrophy caused by heavy metal ions.

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Sažetak

GLIKOGEN U MIŠIĆIMA ŠTAKORA OTROVANIH IONIMA METALA

U skeletnim mišićima štakora koji su tretirani solima žive, kadmija, cinka, bakra, molibdena, olova, kobalta i mangana određen je sadržaj glikogena. Unos ovih metala doveo je do osiromašenja mišića glikogenom, osim u slučaju bakra i cinka. Raspravlja se o uzrocima i značaju promjena koje je trovanje metalima izazvalo u organizmu štakora.

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PROMJENE KRVNIH LOZA RADNIKA IZLOŽENIH OTAPALIMA SA SADRŽAJEM BENZENA *

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Ispitane su vrijednosti krvnih elemenata u 51 radnika u procesu bojenja šivaćih strojeva, od kojih 17 muškaraca i 34 žene, prosječne starosti 35 godina, duljine izloženosti otapalima sa sadržajem benzena 11,8 godina. Radnici su do godine 1981. bili izloženi vrijednostima benzena ispod MDK, a otada nadalje benzena i homologa nema u sastavu otapala. Pri duljoj izloženosti benzenu utvrđen je značajan pad broja leukocita na račun neutrofila. U istih radnika uspoređene su vrijednosti krvnih loza izmjerene 1981. i 1986. godine te je ustanovljeno da je nakon prestanka izloženosti benzenskom otapalu, iako su vrijednosti bile ispod MDK, porastao broj elemenata svih krvnih loza, od čega su vrijednosti eritrocita i trombocita porasle značajno ($P < 0,05$, odnosno $P < 0,01$).

Poznato je da izloženost benzenu izaziva oštećenja u prvom redu na krvnim lozama (1). Toksičnost benzena je ovisna o metabolizmu (1, 2), brzini ulaska otapala u pojedina tkiva i brzini otpuštanja, odnosno distribuciji otapala nakon što dospije u krvni optok (3, 4). S obzirom na to da koštana srž pripada skupini »masnih tkiva« te da je topljivost benzena i toluena u masnim tkivima 30 – 50 puta veća nego u ostalim, ta tkiva dulje u tijelu zadržavaju benzen i sporije ga eliminiraju (4, 5).

Svrha je ovog ispitivanja bila utvrditi: da li pri vrijednostima ispod maksimalno dopuštenih koncentracija (MDK) postoji depresija krvnih loza i da li nakon eliminacije inkriminirane nokse postoji tendencija oporavka krvnih elemenata.

ISPITANICI I METODE

Ispitivanje je obuhvatilo 51 radnika u procesu bojenja šivaćih strojeva, prosječne dobi 35 godina, od kojih 17 muškaraca i 34 žene. Prosječna dužina izloženosti benzenovim

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otapalima iznosila je 11,8 godina. U okviru periodičkih pregleda radnika tvornice »V. Bagat« mjerena je brzina sedimentacije, broj eritrocita, količina hemoglobina, retikulocita, leukocita, neutrofila i trombocita. Dobiveni podaci statistički su obrađeni tako da su izračunate aritmetičke sredine i dobivene standardne devijacije, a značajnost razlika je testirana t-testom (6). Prije ocjenjivanja radne okoline, a na osnovi ranijih ispitivanja (prije 10-ak godina) koja je izvršila Škola narodnog zdravlja »Andrija Štampar« u Zagrebu pretpostavilo se da su u radnoj sredini prisutni benzen i toluen. To je mjerenjem i potvrđeno. Sastav boje i otapala u njima nije bio označen na originalnim pakovanjima te su vrijednosti dobivene mjerenjem organskih otapala u radnoj prostoriji (tablica 1).

REZULTATI I RASPRAVA

Do godine 1981. ispitanici su obavljali poslove pri koncentracijama organskih otapala prikazanima na tablici 1.

Tablica 1.

Usporedba koncentracija para organskih otapala u bojadisaonici i maksimalno dopuštene koncentracije (MDK)

Otapala	Bojadisaonica mg/m³	MDK mg/m³	MDK ppm
Etil acetat	150	200	29
Aceton	260	800	336
Toluen	177	300	—
Benzen	40	50	15

Kao što je vidljivo na tablici, niti jedna koncentracija organskih otapala nije prelazila maksimalno dopuštene vrijednosti. Prosječne vrijednosti krvne slike u radnika s različitim duljinama izloženosti otapalima sa sadržajem benzena navedene su na tablici 2.

Iz tablice je vidljivo da je kod radnika duže izloženih otapalima nađen statistički značajan pad broja leukocita (na račun neutrofila), kao i povećanje broja retikulocita. Na tablici 3. uspoređene su vrijednosti krvnih loza ispitanika koji su sistematski pregledani i laboratorijski obrađeni 1981. i 1986. godine.

Iz tablice 3. očito je da su povećane vrijednosti krvnih elemenata svih krvnih loza nakon prestanka ekspozicije radnika otapalima. Do toga je došlo nakon određenih izmjena u tehnološkom postupku i promjene boje. (Vrijednosti su uskoro ponovno potvrđene određivanjem fenola u urinu u šest najeksponiranijih radnika u Kliničko-toksikološkom laboratoriju Instituta za medicinska istraživanja i medicinu rada u Zagrebu.) Povećanje za leukocitnu lozu nije statistički značajno, dok je za eritrocitnu i trombocitnu značajno ($P < 0,05$, odnosno $P < 0,01$). Prosječne vrijednosti

Tablica 2.

Prosječne vrijednosti krvne slike radnika koji rade u bojadisaonici s obzirom na dužinu izloženosti benzenovim otapalima

	Izloženost ($\bar{X} \pm SD$)		t-test	P
	< 10 godina	> 10 godina		
Leukociti (x 10^9/L)	5,91(1,35)	4,99(1,57)	2,19	< 0,05
Eritrociti (x 10^{12}/L)	4,71(0,50)	4,76(0,52)	0,34	n.s.
Hemoglobin	147,07(20,04)	151,27(15,67)	0,82	n.s.
SE	9,5(6,2)	7,0(6,8)	1,3	n.s.
Trombociti (10^9/L)	262,53(31,35)	268,37(28,61)	0,67	n.s.
Retikulociti (10^9/L)	4,73(1,97)	6,52(2,51)	2,46	p < 0,05
Neutrofili (x 10^0/L)	3,44(1,1)	2,75(1,11)	2,18	p < 0,05

Tablica 3.

Promjene prosječnih vrijednosti krvne slike u istih radnika nakon uklanjanja benzenovih otapala

	Sistematski pregled		Smjer promjena	P
	1981 ($\bar{X} \pm SD$)	1986 ($\bar{X} \pm SD$)		
Leukociti	4,9(1,41) 29	5,14(1,38)	povećanje	n.s.
Eritrociti	4,65(0,39) 26	4,79(0,53)	povećanje	p < 0,05
Hemoglobin	151,14(13,02) 29	148,28(17,87)	smanjenje	p < 0,05
SE	8,45(7,08) 29	7,52(6,78)	smanjenje	n.s.
Trombociti	228(33) 26	265(28)	povećanje	p < 0,01

broja leukocita, eritrocita, trombocita, retikulocita i neutrofila te hemoglobina i brzine sedimentacije bile su u granicama normale. S obzirom na dob i spol ispitanika nije bilo razlike u vrijednosti krvnih elemenata.

ZAKLJUČAK

Na temelju dobivenih rezultata vidljivo je da je duljina izloženosti utjecala na leukocitnu lozu i dovela do smanjenja broja leukocita na račun neutrofila u radnika koji su bili dulje izloženi otapalima s većim sadržajem benzena. Utvrđeno je da je nakon prestanka izloženosti benzenu došlo do regeneracije eritrocitne i trombocitne loze. Dobiveni rezultati upućuju na to da i koncentracije ispod maksimalno dopuštenih po našem standardu uzrokuju promjene na pojedinim krvnim lozama. Ovi rezultati idu u prilog zahtjevu da se pristupi razmatranju izmjene važećih MDK.

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Summary

CHANGES IN THE BLOOD COMPONENTS OF WORKERS EXPOSED TO SOLUTIONS CONTAINING BENZENE

Testing of blood elements was carried out on a sample of 51 semiskilled workers employed in a sewing machine dye-works and exposed to the effect of solutions containing benzene for a period of 11.8 years. The sample consisted of 17 men and 34 women, the average age being 35 years. Up to 1981 values of benzene in the solution were under the maximal allowable concentration

(MAC). From 1981 the injurious agents and their homologues were no longer present in the solution. The results of testing show a significant drop in the number of leukocytes in workers exposed to the effect of benzene for a long period of time. Comparison of blood components was carried out in the same workers during 1981 and 1986. It was found that after exposure to the solution containing benzene ceased (although under MAC), the number of elements of all blood components increased, particularly the values of erythrocytes and thrombocytes ($P < 0.05$ and $P < 0.01$ respectively).

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