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Differential Accumulation of Thallous Ion by Diverse Rabbit and Rat Muscles

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In the past, thallium had been thought to produce mainly acute intoxications. However, there is a growing concern about the possibility of chronic intoxications as a result of environmental contamination produced during industrial processes that involve thallium-contaminated materials (Zitko et al.,1975, Brockhaus et al., 1981; Gorbauch et al.,1984). To determine the severity of both acute and chronic intoxications the total body load is more significant than blood or plasma concentrations due to the thallium uptake kinetics (Talas and Wellhner,1983; Morales-Aguilera and Careaga-Olivares, 1990). In this context, numerous authors have quantified the in vivo accumulation of Tl^+ in skeletal muscles (Rauws,1974;Talas and Wellhöner,1983). All of them have implied that skeletal muscle in other species accumulate Tl^+ in a manner similar to that described by Rauws (1974) in the rat. It is also a general practice to take samples from only one muscle, i.e. quadriceps, and to generalize the concentration data to the whole skeletal musculature. However, in the course of a series of studies on the pharmacokinetics of Tl^+ in the rabbit 0-90 minutes after injection (Morales-Aguilera and Careaga-Olivares,1990) we detected differences in the percent of the dose of Tl^+ taken up by some rabbit muscles as compared with the data of Rauws (1974). We have therefore studied the accumulation of Tl^+ in several skeletal muscles of rats and rabbits at different times after the oral and intravenous administration of thallium sulphate. We found some differences between the two species, as well as a different temporal pattern of accumulation by diverse muscles. The accumulation by the diaphragm resembled that of cardiac muscle in both species.

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MATERIALS AND METHODS.

Twelve New Zeland male rabbits (3.2 þ 0.2 kg; mean þ S.D.) and 12 Sprague-Dawley male rats (373 þ 35 g; mean þ S.D.) were given 10 mg/kg of Tl^+ (12.35 mg/kg of Tl_2SO_4). The rabbits received their dose in aqueous solution through the marginal ear vein, the rats by means of an esophageal cannula. The oral route in the rat was selected for convenience and on the basis of the similar blood levels of thallium in the rat 2 h after oral administration and 1.5 h in the rabbit after i.v. injection of a similar dose(See Table 1). Six of the rabbits were sacrificed 1.5 h and 6 at 24 h after administration. Six rats were sacrificed 2 h and six 24 h after administration. Blood samples were taken from all the animals within 5 minutes before sacrifice. All animals were anesthetized by means of a sodium pentobarbital i.v. (rabbits) or i.p. (rats) injection (35mg/kg) and were exsanguinated through the left ventricle. The following muscles were obtained from the rabbits : rectus abdominis, adductor magnus, posterior trapezius, diaphragm and left ventricle; from the rats rectus abdominis, extensor digitorum longus, quadriceps, soleus, diaphragm, and left ventricle. These muscles were chosen in an effort to obtain a wide sample of muscles with diverse functions and metabolic types in both species (for references see the review by Pette and Vrbov ,1985) since to reduce the choice to a few muscles had the limitation that none is really as homogeneous in metabolic and morphological fiber characteristics as formerly believed (Rosser et al., 1992).

Thallium concentrations in blood and muscles were measured with an atomic absorption spectrophotometer (Perkin Elmer, 5000) after acid digestion. The detection limit of our procedure is 0.1 ug/ml.

The concentration data were analyzed by two-factor analysis of variance [two way ANOV (Zar,1974)].

RESULTS AND DISCUSSION.

All the control muscles and other tissues tested had 0 thallium concentration confirming that none of our animals were previously exposed to the metal.Table 1 presents the Tl^+ concentrations found in all experiments as well as the F and p values found by two way ANOV. Figure 1 depicts the comparison of two skeletal muscles with similar functions (rectus abdominis and diaphragm) and the left ventricle in both species.

Table 1. Tl^+ concentration in rabbit and rat muscles (µg / g wet weight)

Rabbit Muscles	1.5 h	24 h	F **	P **
Diaphragm	69.9 ± 11.0	7.8 ± 1.0	184.0	< 0.001
Left Ventricle	50.0 ± 7.2	10.1 ± 3.0	589.8	< 0.001
Posterior Trapezius	22.1 ± 2.7	8.6 ± 1.5	94.0	< 0.001
Adductor Magnus	12.6 ± 2.4	8.9 ± 2.0	7.3	N.S.
Rectus Abdominis	11.5 ± 0.6	7.7 ± 2.8	8.98	N.S.
Blood *	2.1 ± 0.4	0.5 ± 0.1	76.7	< 0.001
		n = 6		
Rat Muscles	**2.0 h**	**24 h**		
Diaphragm	31.5 ± 7.1	16.2 ± 6.8	26.1	< 0.01
Left Ventricle	24.3 ± 3.9	9.7 ± 2.9	79.3	< 0.001
Soleus	17.2 ± 5.5	11.2 ± 3.7	6.3	N.S.
Quadriceps	6.0 ± 1.3	16.3 ± 4.8	31.1	< 0.005
Extensor digitorum longus	5.6 ± 2.5	9.7 ± 1.4	16.7	< 0.02
Rectus Abdominis	5.5 ± 1.6	15.5 ± 3.5	116.4	< 0.002
Blood *	2.3 ± 0.9	0.7 ± 0.05	21.6	< 0.01
		n = 6		

All values are mean ± S.D.
* µg / ml blood.
** Calculated by two way ANOV.

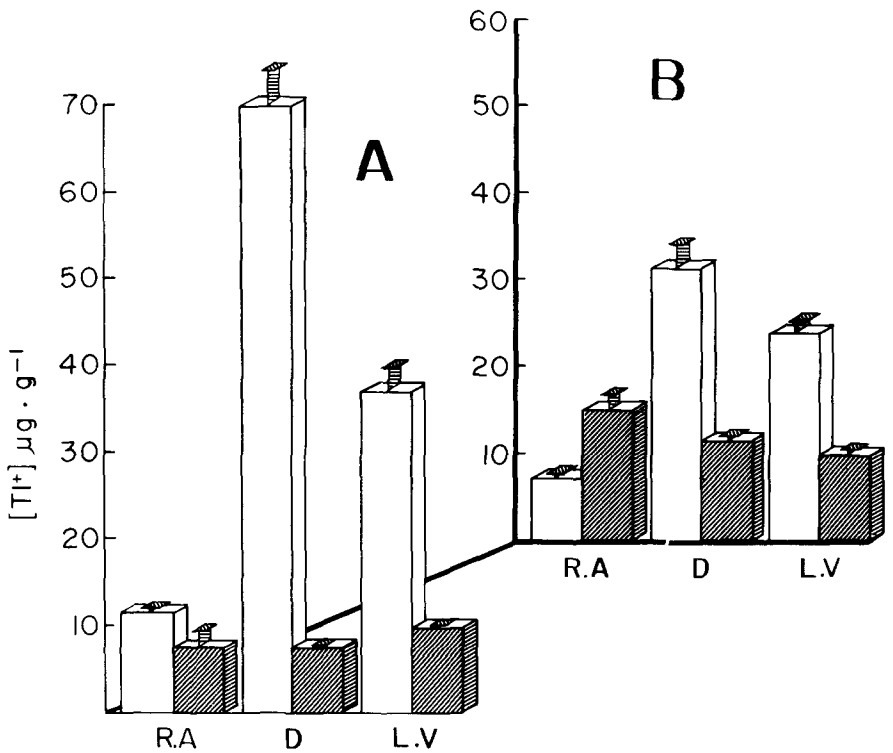

Figure 1. Tl^+ concentration (µg/g wet weight) in rectus abdominis, diaphragm, and left ventricle of rabbit (A) and rat (B) at different times (1.5 or 2 h ,light bars, and 24 h, dark bars) after Tl^+ administration. Values are means with S.E.Ms. (n = 6).

Table 1 illustrates that not all muscles follow the same pattern of accumulation. In the rats quadriceps, extensor digitorum longus, and rectus abdominis reached higher Tl^+ concentrations 24 h after administration than at the end of the 2 h period. In the rabbits posterior trapezius accumulated more thallous ion 1.5 h after injection than 24 h after it, whereas adductor magnus and rectus abdominis Tl^+ concentrations 1.5 h and 24 h after administration were not significantly different. The rat soleus also did not show any significant difference. However, the diaphragm and the left ventricle in both species showed very significant differences with a higher concentration of Tl^+ in the first hours than 24 h after administration.

Figure 1 depicts the patterns of Tl^+ accumulation found in muscles with similar functions in both species. Toxicologists have implicitly accepted that Tl^+ uptake by skeletal musculature is homogeneous, based on concentration data from a few muscles. It is also customary to calculate the contribution of skeletal muscle Tl^+ to the total muscular mass, estimated as 30%-40% of body weight (Rauws,1974;Talas and Wellhner,1983). The results reported here indicate that this procedure could be correct when Tl^+ muscle concentrations are determined 24 h after administration (at least in the rat and rabbit) but it is not reliable when Tl^+ muscle concentrations are determined closer to the time of administration. Besides the different temporal pattern of Tl^+ accumulation by different muscles our data are also suggestive of a species difference between rats and rabbits. It could be argued that we use different routes of administration in the two species, but our results with oral administration to the rat are similar to the results of Rauws (1974) with the quadriceps and with intravenous administration of radioactive Tl^+. Furthermore, preliminary results in our laboratory with oral administration to rabbits have shown that the temporal pattern of Tl^+ accumulation in skeletal muscles is similar to the pattern after intravenous injection.

The purpose of this study was not to determine the kinetics of the uptake but to compare the relative accumulation of thallium at different times after its administration. It is also pertinent to note that the accumulation in the diverse muscles included in this study does not seem to be dependent on the blood or plasma concentration of Tl^+ since that concentration was very low both at 1.5 h and 2 h after the intravenous and oral administration respectively (Table 1). Our acute pharmacokinetics studies have also shown that the fall in blood or plasma Tl^+ concentration could be explained by a mammilary open tricompartamental model and that 30 minutes after injection the concentration in blood is very low, i.e. muscles must take up Tl^+ in the first minutes with a high influx constant and lose it along many hours with a low efflux constant (Morales-Aguilera and Careaga-Olivares, 1990). These data confirm previous studies on the fast disappearance of the thallous ion from the blood. As to the plasma concentrations, Careaga-Olivares and Morales-Aguilera (1990) have found that in the range of blood concentrations herewith reported the ratio of cell packet/plasma is approximately 2:1. Thus, plasma concentrations (and implicitly extracellular fluid concentration) are too low to significantly influence the values found by us in the different muscles. The

very low plasma concentration reached in approximately 30 minutes after administration (Careaga Olivares and Morales-Aguilera, 1990; Rauws, 1974) rules out any passive equilibrium between muscles and extracellular fluid at the times tested by us. Asanoi et al. (1992) have reported that Tl-201 activity in human legs is increased during mild to moderate exercise mainly by a decrease in vascular tone and Caluser et al. (1992) found blood flow is an important factor for Tl+ uptake by bone and soft tissues tumors but that it is not the only factor. In our experiments the rabbits were immobilized during the first 1.5 h after Tl+ injection and there was not evidence of increased blood flow in the muscles. On the other hand,the amount of thallium-contaminated blood remaining in the muscles after excision could not be great since the animals were exsanguinated before the maneuver. Furthermore, the pentobarbital anesthesia greatly diminishes the sympathetic efflux to the capacitance vessels (Chien,1971).

An interesting aspect of our results is the very high Tl^+ concentration in the diaphragm in the first hours after administration. In the case of the rat, the diaphragm Tl^+ concentration 2 h after administration was two times higher than the concentration 24 h afterwards ($p < 0.01$). This pattern is similar to the pattern of the left ventricle. In the rabbit not only did the diaphragm follow that pattern but in all the other muscles tested the concentration of Tl^+ was higher 1.5 h after injection that the concentration found at 24 h, although the diaphragm showed a larger difference (8-9 fold; $p < 0.001$) and was again comparable to the left ventricle. The pattern of the left ventricle is already known since the fast uptake by cardiac tissues has been extensively studied by different authors (Okada et al,1982;McCall et al ,1985).

On the basis of our results alone, it is difficult to explain the case of the diaphragm in both species. Possibilities we favor include : Thallous ion is taken up differently by skeletal muscles depending on the metabolic activity of their fibers with the diaphragm of both species having a very high content of fibres with a high ATPase activity (so-called "red" fibres).In fact, Close (1972) reported that the rat diaphragm has at least 60% "red" fibres, 20% "white" fibres and 20% intermediate ones. "Red" fibres are richer in ATPase and mitochondria and have wider Z-lines than "white" fibres (Close,1972). These intracelllular components would have a high affinity for Tl^+, since normally they bind K^+ , and Tl^+ substitutes for K^+ in structures rich in this ion, even stoichiometrically (Ling,1977).

A similar explanation could also partially apply to other rabbit muscles since the high affinity for Tl^+ of the afore mentioned intracellular structures in mammalian muscle was confirmed by Edelmann (1984) who visualized them by means of electron microscopy and cryosectioning. Other possible explanations for our results are: It is possible that after the initial high uptake the activity per se causes an increase in thallium efflux, as described by Mullins and Moore (1960) in frog sartorius in vitro ,or similar to the potassium efflux in human fibres in vivo during exercise described by Sjgaard (1990). It is also possible that differences in capillary densities in the diverse muscles influentiate the thalliun uptake.

The possibilities suggested here are testable, and further studies are being conducted in order to elucidate the points raised by the present study. These results and related ones might be of some value to toxicologists interested in Tl+ and other heavy metals.

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