Role of Submaxillary Glands in Radiomanganese Metabolism

Liver is the main excretory and regulatory organ involved in manganese metabolism[1], but there are other less important excretory routes[2,3]. The purpose of this study was to determine the role of rat submaxillary glands in the metabolism of the metal.

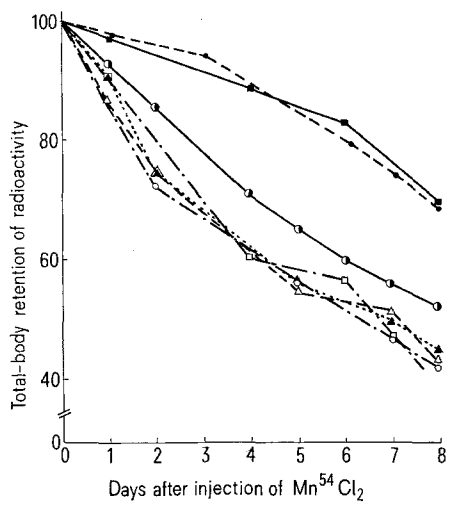
Twelve Double Strain male rats, weighing 200–300 grams, were fed ad libitum with Purina Laboratory Chow (Protinal). 50 mg of $MnCl_2$ per 100 ml of distilled demineralized water were offered ad libitum to 6 of these rats. No manganese was added to the water of the controls. 3 months later, 1 μC of carrier free $Mn^{54}Cl_2$ (Amersham/Searle, Illinois) in 0.5 ml of normal saline was injected into the dorsal vein of the tail of each animal, and body radioactivity was recorded every other day in an Armac Scintillation Counter (Packard Instrument Co., La Grange, Illinois). 19 days thereafter, all rats were sacrificed in an ether atmosphere and the radioactivity of their livers, submaxillary glands and gastrointestinal tracts (GI) was measured. The GI tracts were thoroughly washed with normal saline before weighing and counting.

As previously demonstrated[4], manganese loaded animals showed a rapid excretion of Mn^{54}. From the 12th day, there was a tencency in both groups to form a plateau and on the 19th day the total body retention of radioactivity by the manganese loaded and control animals was 20% and 30%, respectively. The radioactivity in submaxillary glands was slightly higher in the overloaded group, while in liver, as has been shown before[1], it was higher in the control (Table I). The same was observed for the GI tract. When radioactivity was expressed in terms of tissue weight, the difference was negligible in the GI tract, whereas it was significant in liver ($t = 6.58$, $0.01 > p > 0.001$). In the submaxillary glands no difference was observed between the 2 groups ($t = 0.96$, $p = 0.4$), but the radioactivity per gram of tissue was almost 11 times higher than in liver for the overloaded animals, and 4 times higher for the controls. These results have 3 possible interpretations: 1. the glands are excretory routes for manganese, but of slow rate; 2. the glands are storage organs for the metal; and 3. they are both excretory and storage organs.

Twenty rats were injected i.v. with 1 μC of $Mn^{54}Cl_2$ each and killed in groups of 2 at regular intervals (Table II). The liver initially incorporated a high amount of Mn^{54} but, in the first 24 h, a loss of about 50% was recorded. This is due to an initial rapid excretion of the metal into the bile and redistribution to other tissues[3]. Since the radioactivity expressed as percentage of total-body radioactivity increased with time in submaxillary glands, it can be suggested that the remaining organs are eliminating Mn^{54} more rapidly. When expressed in cpm/mg of tissue it was almost constant during the 12 days of the experiment.

Five rats were anesthesized i.p. with nembutal and submaxillary glands extracted. In another group of 5 animals the common bile duct was ligated, and in other 5 rats, submaxillary glands were extracted and the common bile duct ligated. Each group had its own sham-operated control of 3 animals. A final group of 3 animals was not operated. Each rat was injected i.v. with 1 μC of $Mn^{54}Cl_2$, and body radioactivity decay determined.

The Figure shows no difference between the rats without submaxillary glands and their controls. The same response was observed between the group with bile duct ligated and the group with submaxillary glands extracted and bile duct ligated. This might indicate that the glands are not excretory organs or that the excretion is not high enough to reveal any difference. The non-operated animals had a lower rate of decay than the controls[1]. On the 8th day the rats were killed, the livers and submaxillary glands extracted, and their radioactivity determined. In animals with the bile duct ligated, the liver had a radio-



Total-body retention of Mn^{54}. Conditions of the experiments are described in the text. ▲....▲, submaxillary glands extracted; △----△, control; ■—■, bile duct ligated and submaxillary glands extracted; □-.-.-□, control; ◑—◑, non-operated animals; ●----●, bile duct igated; ○-.-.-○, control.

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Table I. Organ distribution of Mn^{54} in rats overloaded with $MnCl_2$.

Groups	Total-body radioactivity (%)			Radioactivity per g of tissue (total-body radioactivity, %)		
	Liver	Submaxillary glands	Gastrointestinal tract	Liver	Submaxillary glands	Gastrointestinal tract
Rats overloaded with $MnCl_2$	4.03 ± 0.69	2.00 ± 0.40	5.33 ± 1.53	0.38 ± 0.08	4.00 ± 0.80	0.20 ± 0.08
Control	9.33 ± 0.51	1.49 ± 0.41	8.31 ± 0.50	0.75 ± 0.00	2.98 ± 0.82	0.25 ± 0.02

Conditions of the experiment are given in the text.

Table II. Mn^{54} incorporation in liver and submaxillary glands at different time intervals

Time after injection of $Mn^{54}Cl_2$ (h)	Liver			Submaxillary glands		
	Injected radioactivity (%)	Total-body radioactivity (%)	cpm/mg of tissue	Injected radioactivity (%)	Total-body radioactivity (%)	cpm/mg of tissue
1	31.15	29.43	41	0.93	0.88	26
2	25.23	23.83	35	0.95	0.91	32
8	21.58	19.25	26	1.07	0.95	23
24	14.43	18.40	19	0.81	1.04	28
48	16.60	24.90	17	0.78	1.17	22
72	17.73	29.19	18	0.99	1.62	33
96	15.09	23.41	13	0.94	1.46	25
120	10.31	16.44	10	1.06	1.70	29
192	7.85	16.95	6	0.56	1.23	12
288	4.93	15.08	2	0.59	1.79	17

Conditions of the experiment are given in the text.

Table III. Radioactivity retention of liver and submaxillary glands subjected to the conditions described in the text

Group of animals	Total-body radioactivity (%)		Radioactivity per g of tissue (Total-body radioactivity, %)	
	Liver	Submaxillary glands	Liver	Submaxillary glands
Bile Duct Ligated	54.30 ± 3.0	1.05 ± 0.2	3.53 ± 0.0	2.44 ± 0.0
Control	12.57 ± 1.4	1.53 ± 0.3	1.09 ± 0.0	3.73 ± 0.0
Bile duct ligated and submaxillary glands extracted	47.25 ± 6.7	—	3.89 ± 0.0	—
Control	13.97 ± 2.7	1.46 ± 0.0	1.46 ± 0.0	3.40 ± 0.0
Submaxillary glands extracted	12.27 ± 1.5	—	—	—
Control	11.16 ± 1.2	1.37 ± 0.56	—	—

Table IV. Subcellular distribution of Mn^{54} Cl_2 in rat submaxillary glands

Fraction	Radioactivity (cpm)	Total radioactivity (%)
Homogenate	9800	—
Supernatant	3407	35
Microsomal	2715	28
Mitochondrial	1694	17
Amilase granules	1191	12
Nuclear	785	8

Experimental details are described in the text.

activity more than 4 times higher than the controls, while submaxillary glands had a slightly higher, though insignificant, radioactivity in the controls (Table III). Similar results were observed in the livers of the group with the bile duct ligated and submaxillary glands extracted. When only the submaxillary glands were extracted, there was on difference in the radioactivity found in the livers of the problem and control groups. This suggests that if submaxillary glands excrete Mn, they do not increase that function when the main excretory route is blocked.

Two groups of 3 rats were injected i.p. with 20 μC of $Mn^{54}Cl_2$ and 16 h later the submaxillary glands were extracted and the subcellular distribution of the metal studied as previously described[5]. Each resulting fraction was extracted and counted. Manganese was located in all particulate fractions, especially in the microsomal and mitochondrial fractions (Table IV).

The presence of Manganese in mitochondria and its importance in oxidative phosphorylation have been reported[6,7]. The large number of differently located enzymes that require manganese for activation could explain the observed ubicuity of the metal in the intracellular organelles of submaxillary glands. The stability of the Mn^{54} present in glands could indicate binding of the metal to proteins of a very slow turnover, but the nature and mechanism of control of this binding, if it exists, remains unknown[8].

Zusammenfassung. Die submaxillaren Drüsen der Ratte, die Mn^{54} inkorporieren, schienen sich wie Metall speichernde Organe zu verhalten. Die Radioaktivität der Drüsen bleibt einige Tage konstant, da sie offenbar ein Metall-Protein-Komplex mit langsamer Umsatzrate bindet. Das Isotop verteilt sich in allen Zellfaktoren, besonders in Mitochondrien und Mikrosomen.

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