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VARIATIONS IN RAT LIVER CALCIUM AND MAGNESIUM*

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Abstract—Significantly higher magnesium concentrations were found in the livers of young, healthy, female rats than in the livers of normal males of the same age. The calcium concentration in the liver from femae rats also tended to be higher than for males. Rats given thioacetamide or carbon tetrachloride had liver calcium concentrations up to sixty times normal values. Necrotic liver produced by yellow phosphorus administration had about three times the calcium concentration of normal liver. Fatty infiltration induced by choline deficiency produced no change in the calcium level of the liver. The magnesium concentration in the liver was affected to a far less extent by these treatments than the calcium.

INTRODUCTION

IN 1956, Gallagher, *et al.*[1] observed that the liver calcium concentration of adult male rats showed increases of up to fortyfold after one subcutaneous injection of thioacetamide. Subsequently, Thiers and Reynolds[2] have indicated that carbon tetrachloride administration increased the Ca level of rat liver mitochondria. These results indicated that liver damage might result in increases in liver calcium concentration.

It was thought that these increases in Ca concentration might occur in all types of liver damage. In order to test the generality of this phenomenon and the specificity of the cell membrane to the increases in Ca transport into the cell, both the Ca and Mg levels of rat liver in various types of liver damage have been examined.

METHODS

Animals. Young adult male rats, unless otherwise indicated, were used. They were fed commercial rat cubes both before and during the administration of the hepatic toxins, except for the animals made choline deficient. These animals received a diet described by Kratzing and Windrum.[3] Some choline deficient animals received ethyl trichloroacetate which was injected subcutaneously at 0·075 ml/100 g body weight once daily for 5 days. Animals were killed by decapitation, the livers removed, weighed and analysed.

Toxic agents. Yellow phosphorus in peanut oil (5 mg P/ml) was given by intraperitoneal injections. Carbon tetrachloride was similarly injected as a 50 per cent solution

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in peanut oil. Thioacetamide was injected subcutaneously as a 2 per cent aqueous solution.

Analytical procedures. Calcium was determined by titration with ethylenediamine-tetra-acetic acid (EDTA) using murexide as indicator,[4] and the total calcium and magnesium by titration with EDTA, using Eriochrome Black T as indicator after phosphate had been removed by precipitation with morpholine nitrate. By this method calcium and magnesium have been conveniently estimated with a maximum error of 5 per cent.[5]

Nitrogen was determined by the method described by Francis *et al.*[6]

RESULTS AND DISCUSSION

Normal liver

Livers from sixteen young male rats and sixteen female rats of the same age were analysed for calcium and magnesium. The values are given in Table 1. The results showed that there was no difference in the calcium content per gramme of wet liver between male and female animals. However, livers from female rats had significantly greater amounts of magnesium per g of wet liver ($P<0{\cdot}001$). When the results were expressed in micromoles per gramme of liver nitrogen (μmoles/g) the females had significantly higher values for both calcium ($P < 0{\cdot}05$) and magnesium ($P < 0{\cdot}001$). Variations in the level of calcium in the liver occurred in both sexes. This may have been due to occluded blood although Thiers and Vallee[7] also found a big variation in the calcium content of rat liver even after the livers had been perfused. The concentration of magnesium in the liver was more constant than that of the calcium.

The values obtained for both the calcium and magnesium levels in normal liver are in agreement with those obtained by Thiers and Vallee[7] and Griswold and Pace[8], although neither group reported any difference made by the sex of the rats.

The ratio of liver to body weight in males (4·4 per cent) was greater than in females (3·8 per cent); this sex difference was highly significant ($P < 0{\cdot}001$).

This type of sex differentiation in growth has not been reported previously (see Webster, *et al.*[9]) and may be a characteristic of our colony of rats.

Damaged liver

Liver toxins can be divided into two main classes; those which primarily damage the centrilobular area, and those which first affect the peripheral portions of the liver lobule.[10] With the exception of yellow phosphorus, the toxins used produced centrilobular damage. The changes in liver calcium and magnesium concentrations produced by these agents are shown in Table 1.

Choline deficiency

Macroscopically the livers appeared very fatty. In wet liver the calcium level was not significantly greater than that found in normal animals, but there was a greater concentration of calcium in the choline-deficient livers when expressed on a nitrogen basis ($P < 0{\cdot}01$). In these choline-deficient animals a small decrease in the magnesium concentration of wet liver and an increase in the magnesium content per gramme of nitrogen occurred. It is considered that these changes are non-significant and are a consequence of the presence of fat in these livers. Ethyl trichloroacetate has been found by Kratzing and Windrum[3,11], to remove the excess lipid which accumulates

TABLE 1. THE CALCIUM AND MAGNESIUM CONCENTRATION IN DAMAGED LIVER

Treatment	Dosage	Sex	No. of animals	Mean body wt. (g)	Ca concentration		Mg concentration	
					μ moles/g wet liver	μ moles/g N	μ moles/g wet liver	μ moles/g N
untreated normal	—	M	16	89	0·76 ± 0·06*	23·6 ± 1·9	9·18 ± 0·22	289 ± 7
untreated normal	—	F	16	77	0·91 ± 0·08	33·0 ± 2·9	10·20 ± 0·10	360 ± 13
choline deficiency	3½ weeks deficiency	M	6	95	0·86 ± 0·10	33·6 ± 3·2	8·61 ± 0·28	341 ± 8
choline deficiency + ethyl trichloroacetate	5 × 0·075 ml/100 g body weight	M	5	79	0·73 ± 0·17	26·2 ± —	9·24 ± 0·23	321 ± —
yellow phosphorus	1 × 0·5 mg	M	6	Ca 90	0·80 ± 0·12	28·3 ± 4·1	8·62 ± 0·45	306 ± 16
	4 × 0·1 mg	M	5	Ca 100	1·98 ± 0·37	75·9 ± 12·4	7·83 ± 0·59	301 ± 15
carbon tetrachloride	3 × 0·05 ml	M	10	99	5·54 ± 2·6	226 ± 108	8·48 ± 0·18	334 ± 9
	3 × 0·2 ml	M	4	146	30·0 ± 7·3	1379 ± 341	8·20 ± 0·76	369 ± 31
thioacetamide	1 × 20 mg/100 g body weight	M	6	137	45·0 ± 2·5	1716 ± 95	13·43 ± 0·63	511 ± 20

* Standard error of mean.

in the liver of choline-deficient rats. A group of choline-deficient rats was given five daily doses of ethyl trichloroacetate at a dosage which was previously found effective in clearing the liver of excess lipid. At death the livers of these rats had a normal appearance with no excess lipid. The calcium and magnesium concentrations in the liver were found to be similar to those found in normal animals.

Yellow phosphorus

Six rats were given one dose of 0·5 mg phosphorus. This large dose of phosphorus killed the animals in a few hours but no significant changes from normal values in the liver calcium or magnesium concentrations were observed. When rats were given 0·1 mg of phosphorus a day for 4 days, it was found that the liver calcium concentration had increased to about three times that of the normal, although the magnesium concentration remained unaltered.

Carbon tetrachloride

Three daily doses of carbon tetrachloride (0·05 ml in oil) increased the mean calcium concentration in the liver about seven times. The individual animal responded to a different extent to the toxin and hence the large standard error of the mean for this group. In an attempt to produce a more uniform response, four rats were given four times the original dose over the same period of time. In this experiment the calcium level was increased to about forty times the normal value but the variation was still large. In the same animals the magnesium concentration in the liver remained about the same as that for normal animals.

Thioacetamide

Gallagher *et al.*[1] first reported an increase in liver calcium after thioacetamide administration. We confirmed their results and found that one injection of 20 mg thioacetamide per 100 g body weight increased the liver calcium level approximately sixty-five times. However, the magnesium concentration in these livers was only about 50 per cent higher than the value for normal animals.

The toxins tried, regardless of the site of action caused an increase in calcium concentration when given in suitable doses.

The magnesium content of the liver is characterized by extreme stability when compared to the calcium content. After thioacetamide administration when the calcium concentration in the liver had increased over sixty times, the magnesium concentration only increased by about 50 per cent. For the other treatments no such increases in magnesium concentration were observed. It is unlikely that the bulk of calcium accumulating in necrotic tissue is in ionic form, but is most likely bound either to structural molecules or to a metabolic product such as citrate or inorganic phosphate. Gallagher *et al.*[1] found that the concentrations of citrate and calcium did not appear to be directly connected. The increases in total calcium may lead to an increase in ionic calcium which might then act as an ionic antagonist to magnesium in the activation of some cellular enzymes with resulting changes in the activity of some enzyme systems. For example, Lehninger[12], using a particulate fraction of rat liver, found that calcium ions were a potent inhibitor of oxidative phosphorylation.

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