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Swelling of Heart and Liver Mitochondria from Magnesium Deficient Rats and Its Reversal.* (26924)

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(Introduced by F. J. Stare)

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Heart mitochondria isolated from rats fed a magnesium deficient diet have a low P/O ratio(1). The uncoupling effect of thyroxine on oxidative phosphorylation is reversed by dietary magnesium(2). However, it is not clear whether the effect of thyroxine and magnesium is related to the biochemical or to the physical integrity of the mitochondrial system(3).

This paper presents a study of the swelling of mitochondria of the heart and liver of rats fed a magnesium deficient diet. Of particular interest is the observation that these mitochondria rapidly became swollen, although their magnesium content was not significantly decreased. ATP† reversed the swelling of mitochondria isolated from the heart and liver of both control and magnesium deficient rats. EDTA prevented the swelling of mitochondria isolated from liver(4,5), but did not prevent the swelling of heart mitochondria, which contained almost twice the amount of magnesium found in liver mitochondrial fraction.

Methods. Young albino male rats, weighing 45 to 50 g were fed diets containing the following constituents per 100 g of diet: casein (purified) 20; sucrose 30; wheat starch 34; salt-mixture of Jones Foster(6) with $CaCO_3$ and $MgSO_4$ removed, 3.0; vitamins A, D and E mixture 1.0; soybean oil 10.0; and $CaCO_3$ 2.0. The vitamins A, D and E mixture contained 5 g of halibut liver oil, 1 g of α-tocopherol acetate and 34 g of soybean oil. The amounts of the water-soluble vitamins used in the diet have been listed elsewhere(7). Magnesium was added to the control diet in the form of MgO to supply 48 mg of magnesium per 100 g of diet. The magnesium deficient diet contained less than 2 mg % of magnesium.

The rats were housed in individual cages, and fed *ad libitum*. The temperature was maintained at 20 ± 2°C. The rats were weighed twice weekly and observed daily for

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† Abbreviations: ATP, adenosine triphosphate; EDTA, ethylene-diaminetetraacetic acid; Tris, tris (hydroxymethyl) aminomethane; DPN, diphosphopyridine nucleotide.

10 to 12 days for signs of magnesium deficiency. At the end of this period, the rats were sacrificed.

Liver and heart mitochondria were isolated by Lehninger's method(5). The swelling of heart and liver mitochondria was measured spectrophotometrically(5). The standard system was 3.0 ml of 0.25 M sucrose, 0.02 M tris buffer, pH 7.4. An Hitachi spectrophotometer (equipped with thermospacers to maintain temperature in the cuvette carriage chamber at 37°C) was used unless otherwise specified.

The swelling rate of mitochondria was expressed as the reciprocal of the time required to reach one-fourth the maximal extent of swelling as determined by absorbency (called "a reciprocal of quarter time," $\frac{1}{t_{1/4}}$ min.$^{-1}$(5)). Precautions were taken to keep the temperature of the reaction medium constant in order to investigate the difference in rate of swelling between contral rats and magnesium deficient rats. Before adding the isolated mitochondrial suspension kept at 0°, the reaction medium in a Beckman cell was kept in a constant temperature water bath, and the cuvette carriage chamber was kept at a constant temperature by circulating the warm water.

Magnesium and phosphorus concentrations of isolated mitochondria were determined in animals other than those sacrificed for the studies on mitochondrial swelling as follows: The original stock suspension of mitochondria suspended in sucrose solution was centrifuged at 10,000 r.p.m. for 10 minutes. The pellets were homogeneously dispersed in distilled water. One-tenth to 0.25 ml was taken for microkjeldahl analysis of nitrogen. The remainder was deproteinized by addition of 3 volumes of 10% of trichloroacetic acid at 0°C. The supernatant was used for determination of phosphorus and magnesium by methods described elsewhere(8,9). Two rats from each group were used for determination of magnesium and phosphorus in mitochondria, and for determination of the swelling rate of heart mitochondria.

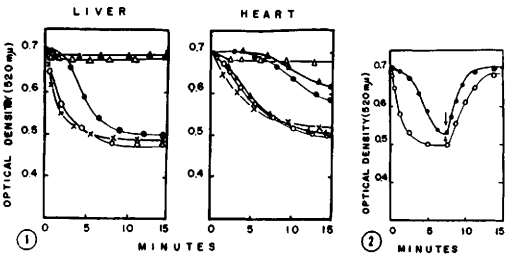

FIG. 1. Rates of swelling of heart and liver mitochondria from rats fed a diet containing 48 mg % magnesium and a diet deficient in magnesium. Measurements were made at 520 mμ, temp. 37°. The following additions were made to 0.25 M sucrose—0.02 M Tris, pH 7.4: ●—● no addition of control rats, ○—○ no addition of magnesium deficient rats; △—△ ATP (0.004 M), ▲—▲ EDTA (0.001 M), ×—× thyroxine (5×10^{-5} M) additions to both of control and magnesium deficient rats. In analysis of liver, 2 rats were used for each point. For heart, one rat was used for each.

FIG. 2. Reversal of mitochondrial swelling in control and magnesium deficient liver by ATP in KCl medium. Measurements were made at the same conditions described in Fig. 1. At zero time: ●—● no addition of control liver mitochondria, ○—○ no addition of magnesium deficient liver mitochondria. At 7.5 min., ATP (0.004 M as a final concentration) was added.

The electron microscopic observations were carried out by the methods described by Palade(10).

The final concentration of L-thyroxine was varied from 1.7×10^{-4} to 5×10^{-5} M.

Results. Liver and heart mitochondria isolated from magnesium deficient rats showed a higher rate of spontaneous swelling than those from controls (Fig. 1, Table I). The rate of spontaneous swelling of heart mitochondria from magnesium deficient rats was much higher at 37° than at 20° (see Exp. No. 3 in Table I).

Effects of thyroxine, ATP, EDTA and DPN on rate of spontaneous swelling. In this experiment thyroxine accelerated the swelling rate of heart mitochondria of the control rat as shown in Fig. 1. The effect of addition of thyroxine was greater in control than in magnesium deficient mitochondria, since thyroxine produced a slight increment or no increment in rate of swelling of mitochondria isolated from magnesium deficient rats. This result was confirmed in the experiment using 0.25 M sucrose, 0.02 M tris buffer medium. The effect of addition of ATP on the swelling rate was determined in

TABLE I. Swelling Rate of Mitochondria $\left(\frac{1}{t_{1/4}\ \text{min.}}\right)$ at 37°C.

Exp.	Heart* Control	Heart* Mg-def.	Liver† Control	Liver† Mg-def.
1	.077	.33	.3	.4
2	.10	.33	.4	1.1
3	.12 (.087)	.33 (.087)	.44 (.10)	.60 (.12)
4	.12	.74	.44 (.06)	.82 (.20)
5	.10 (.076)	.20 (.135)	.46 (.24)	.81 (.50)

( ), values at 20°C.

* Two rats were used for each control and each magnesium-deficient determination in each experiment, No. 1 through No. 5.

† One rat was used for each determination in each experiment.

0.15 M KCl, 0.02 M tris medium. ATP completely prevented the spontaneous swelling of liver and heart mitochondria isolated from both the control and the magnesium deficient rats. ATP also completely reversed the swelling after it had nearly reached the terminal absorbency plateau in a medium of 0.15 M KCl, 0.02 M tris buffer (Fig. 2). This effect was confirmed in the control and magnesium deficient mitochondria. EDTA, which was added to investigate the action of metal chelating agent in KCl-tris medium, showed a protective effect against swelling of liver mitochondria of both control and deficient rats (Fig. 1). Similar observations have been noted by others(4,5,11). However, EDTA did not prevent or reverse the spontaneous swelling of heart mitochondria isolated either from control or magnesium deficient rats. Addition of DPN (final concentration of 0.3 mM) produced no demonstrable effect on rate of mitochondrial swelling under these conditions.

Determination of magnesium and phosphorus in mitochondrial fraction. There was a considerable degree of variation in magnesium and inorganic phosphorus content of mitochondria from rat heart and liver. No statistically significant decrease was found in magnesium in mitochondria of both heart and liver obtained from magnesium deficient rats (Table II). Each determination and S.D. was calculated from 5 runs, each using 2 rats. Magnesium and inorganic phosphorus were determined on the same group of rats.

Demonstration of mitochondrial swelling in vivo. Mitochondria in cardiac muscle of magnesium deficient rats occasionally showed a morphological disorganization of fine structure and a swelling *in vivo* (Fig. 3 and 4).

Discussion. Growth inhibition produced in young rats by addition of thyroxine to the diet is partially overcome by extra magnesium supplements. The amount of magnesium required is related to the amount of thyroxine added(2). The effectiveness of thyroxine in depressing the P/O ratio of oxidative phosphorylation in isolated liver mitochondria depends on the concentration of magnesium ion in the test medium(3). According to Tapley and Cooper(3), it is conceivable that interaction between thyroxine and magnesium may occur at critical sites within the mitochondrial structure.

Dietary magnesium deficiency in rats produces a more marked decrease in P/O ratio of heart mitochondria than of liver and kidney mitochondria(1). The present study indicates that *in vitro* patterns of swelling of mitochondria from rats fed diets deficient in magnesium are similar to the spontaneous swelling in control rats when ATP and EDTA are added, but appear to be different from swelling induced by deficiency of essential fatty acids, in which case ATP does not prevent the swelling(11). The mitochondria of heart and liver from magnesium deficient rats respond very little, if at all, in the presence of thyroxine. *In vitro* swelling of mitochondria isolated from rats fed magnesium defi-

TABLE II. Phosphorus and Magnesium Content of Rat Heart and Liver Mitochondria Expressed in μmoles/g of Mitochondrial Nitrogen.*

Tissue	Group	Magnesium	Inorganic phosphorus
Heart mitochondria	Control rats	467 ± 43†	136 ± 11†
	Mg-def. rats	481 ± 96	144 ± 18
Liver mitochondria	Control rats	303 ± 34	332 ± 62
	Mg-def. rats	313 ± 26	331 ± 55

* Each value is mean and S.D. of 5 experiments of 2 rats each.

† Mean ± S.D.

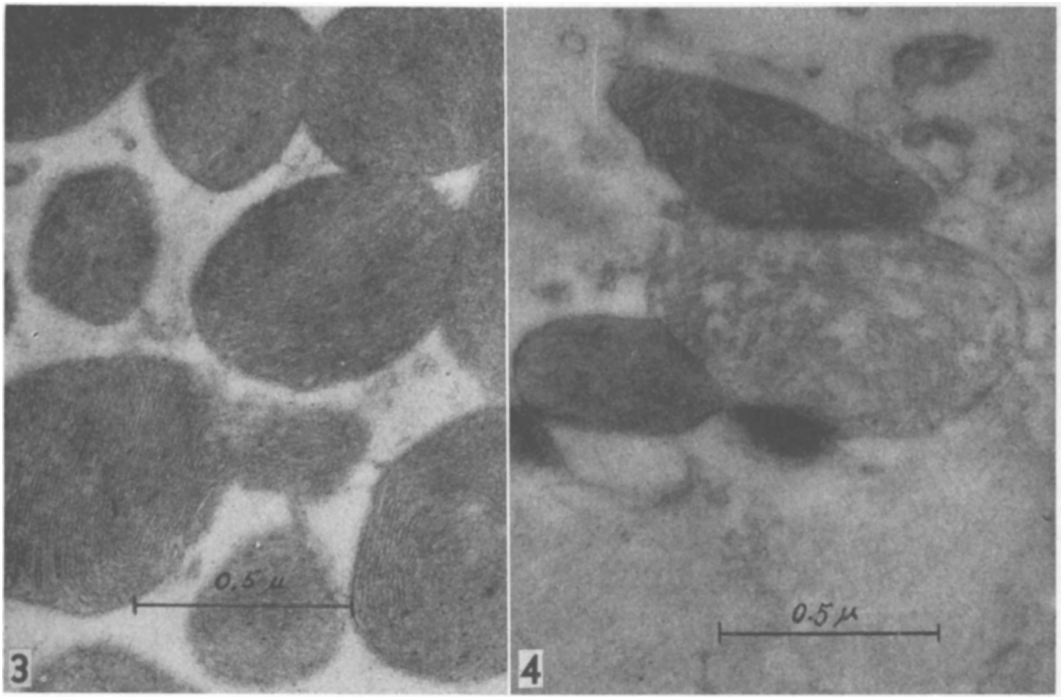

FIG. 3. Mitochondria of myocardium from control rat.
FIG. 4. Mitochondria of myocardium from rats fed a magnesium deficient diet, showing a swelling and disorganization of Cristae mitochondriales.

cient diets has *in vivo* significance, since electron microscopic examination of myocardial sections of magnesium deficient rats shows the mitochondria to be swollen with disorganization of the cristae mitochondriales.

It is unlikely that the higher rate of mitochondrial swelling in magnesium deficiency is due to a decrease in magnesium and/or to an increase in inorganic phosphorus in mitochondria, because magnesium and phosphorus levels were not changed in a statistically significant way. For these reasons, it was assumed that the primary effect of magnesium deficiency in causing a decrease P/O ratio may be an indirect effect mediated by some morphological membrane disorganization. Whether the increased swelling rate of mitochondria isolated from magnesium deficient rats is related to the probable loss of functional DPN as in the case of thyroxine induced swelling(5), or to some other cause such as changes in the calcium or fatty acids content(11), is being investigated further. As a preliminary result obtained in our laboratory, homogenate and mitochondria from the livers of rats fed a magnesium deficient diet for 10 days did not show any deficiency in respiratory control by phosphate acceptor. The relationship between the sensitivity of heart mitochondria to magnesium deficiency, the facts that swelling of heart mitochondria was not prevented by EDTA and that heart mitochondria contain almost twice the amount of magnesium found in liver mitochondria, remain as unsolved problems.

Summary. Swelling of mitochondria isolated from the heart and liver of rats fed a magnesium deficient diet was examined photometrically and by electron microscopy. A rapid swelling of heart and liver mitochondria was produced in rats fed a magnesium deficient diet for 10 days, whereas there was no significant decrease in the magnesium content of the mitochondria. A morphological swelling of heart mitochondria of rats fed a magnesium deficient diet was also demonstrated. Addition of thyroxine produced a slight increment or none in the rate of swelling of mitochondria isolated from deficient rats. ATP reversed swelling of heart and

liver mitochondria from deficient rats; EDTA, however, prevented only the swelling of liver mitochondria. Mitochondria of heart contained twice the amount of magnesium found in liver mitochondria. The mechanism of decrease of P/O ratio of mitochondria from magnesium deficient rats and the physiological significance of magnesium deficiency induced swelling of mitochondria, were discussed.

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Effects of LSD-25 on Food Intake in the Rat.* (26925)

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In an earlier study(1) of certain similarities between the effects of LSD-25 and amphetamine sulfate, a depression of body weight was observed in rats injected daily with 0.50 mg/kg of LSD-25. It seemed logical to suppose that this was the result of depression of food intake since Winter and Flataker(2) reported that at the height of the drug effect their rats did not eat. The present work quantifies this effect of LSD-25 and investigates the possibility of development of tolerance to daily doses of the drug.

Method and results. Experiment I. Thirty-six male albino rats of the Sprague-Dawley strain were trained for 18 days to feed 2 hours a day (2-4 p. m.) on powdered Purina Lab Chow. At the end of this period their mean body weight of 246 ± 3 g was approximately 80% of normal and they were assigned to one of 4 groups on the basis of mean amount of food intake over the last 3 training days. (Mean weights of the 4 groups did not differ significantly). Just before 2 p.m. on the 19th day, one of each of 3 groups was injected IP with one of the following doses of LSD-25: 0.13, 0.26, or 0.52 mg/kg. (LSD-25 was dissolved in Ringer's solution and doses prepared so that all animals received the same amount of fluid in proportion to body weight). The fourth group was injected with a comparable amount of Ringer's solution alone. Immediately after injection the animals were returned to their living cage, given cups of chow, and permitted to feed 2 hours. Four days of no injections and 2-hour feeding followed. On the fifth day each animal was again injected with the same dose level and permitted to feed 1½ hours. After another no-injection, 4-day interval with 2-hour feeding, the animals were again injected with the same doses and permitted to feed for one hour.

The data of this study (Fig. 1), show a depression of food intake as a function of LSD-25 dosage. A repeated measures analysis of variance(3) was run using the data of all 4 groups over the 3 injection days. This analysis yielded the following F ratios: be-

* The authors gratefully acknowledge the assistance of Wallace Clay in collecting data.