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Sarcosomes and Magnesium Deficiency in Ducks

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Reports from this Laboratory have shown that various diets affected the efficiency of oxidative phosphorylation by rat-heart sarcosomes (mitochondria). Sarcosomes isolated from rats fed a diet low in magnesium and supplemented with thyroxine had a low P:O ratio, which was elevated to near normal when dietary magnesium was increased (Vitale, Hegsted, Nakamura & Connors, 1957*a*). Vitale, Nakamura & Hegsted (1957*b*) reported that rat-heart sarcosomes were more susceptible to uncoupling of oxidative phosphorylation by a magnesium deficiency or thyroxine excess than were liver or kidney mitochondria. Vitale *et al.* (1957*c*) later demonstrated that sarcosomes had a lowered P:O ratio when isolated from rats given a purified diet containing cholesterol, cholic acid and 24 mg. of magnesium ions/100 g. of diet as compared with sarcosomes from control rats not given the sterols.

Heart sarcosomes from rat, cat and ox have been isolated, but no work appears to have been done with duck as the source of these respiratory particles. The duck heart, as compared with rat heart, yields more tissue per animal because the heart to body weight ratios are approximately 0.8 and 0.4 % respectively.

The following study is concerned with the effects of a low-magnesium diet on oxygen consumption, oxidative phosphorylation and adenosine-triphosphatase activity of duck-heart sarcosomes.

EXPERIMENTAL

Day-old ducklings weighing approximately 60 g. were grouped in fours and housed in large stock cages. They were fed a commercial chick mash for approximately 5 days before the start of the purified diet, which was fed *ad lib.* for about 8 days. Tap water was present at all times. The purified diet consisted of the following (per 100 g.): glucose, 52.4; casein (purified), 18.0; gelatin, 10.0; Celluflo powder cellulose (The Chicago Dietetic Supply House, 1748-50-52 W. Van Buren Street, Chicago 12, Ill., U.S.A.), 3.0; Jones-Foster (1942) salt mixture with calcium carbonate and magnesium sulphate removed, 5.0; choline chloride, 0.3; calcium carbonate, 1.5; calcium hydrogen phosphate, 1.0; and hydrogenated cotton-seed oil, 10.0. The following vitamins were added (/kg. of diet): 1 g. of a mixture of vitamins A, D and E [prepared by adding 5 g. of Haliver Oil Plain (vitamin A, 60 000 U.S.P. units/g., and vitamin D, 600 U.S.P. units/g.; Abbott Laboratories, North Chicago, Illinois, U.S.A.), and 5 g. of DL- α -tocopherol (Merck and Co. Inc., Rahway, N.J., U.S.A.) to 40 g. of corn oil (Mazola)], 4 mg. of thiamine hydrochloride, 8 mg. of riboflavin, 4 mg. of pyridoxine hydrochloride, 25 mg. of calcium pantothenate, 40 mg. of niacinamide, 1.0 mg. of menadione, 1.0 mg. of folic acid and 0.2 mg. of biotin. Magnesium was added to the diet in the form of magnesium oxide as indicated.

Ducks were decapitated and sections of kidney and heart were fixed in 10 % neutral formalin for microscopic examination. Heart sarcosomes were isolated in various concentrations of sucrose, indicated in individual experiments, by the method of Schneider & Hogeboom (1950). Oxygen uptake and oxidative phosphorylation were determined by

the general procedure of Lardy & Wellman (1952). Warburg vessels contained in a final volume of 3 ml.: hexokinase (35 000 K.M. units/g. at 30°, Nutritional Biochemicals Corp., Cleveland, Ohio, U.S.A.), 2.5 mg.; glucose, 0.02M; cytochrome c, 40 μM; α-oxoglutarate, 6.84 mM; magnesium chloride where indicated, 0.05M; potassium phosphate, pH 7.5, 0.013M; sodium fluoride, 13 mM; adenosine triphosphate, 2.28 mM; potassium chloride, 15 mM; and 0.5 ml. of sarcosomes (5 mg. of protein, average). The final pH of all reaction mixtures was 7.4–7.5. The centre well contained 0.2 ml. of 20% potassium hydroxide. Cold sarcosomes were added to chilled flasks containing all components. After equilibration for 10 min. at 37° with air as the gas phase, the reaction was started when hexokinase and glucose were tipped from the side arm into the main chamber of the flask and continued for 15–20 min. Phosphorus was determined by the method of Taussky & Shorr (1953).

Adenosine-triphosphatase activity was assayed according to Myers & Slater (1957). The adenosine-triphosphatase incubating medium contained: 2,4-dinitrophenol, where indicated, 50 μM; sucrose, 0.05M; tris-acetate buffer, pH 7.0, 0.05M; EDTA, 0.5 mM; potassium chloride, 75 mM; magnesium chloride where indicated, 0.01M; adenosine triphosphate, 2 mM; 0.3 ml. of sarcosomes (3 mg. of protein, average); and sufficient water to make a final volume of 3 ml. The final pH of all reaction mixtures was 6.9–7.0. The mixture was incubated in a metabolic shaker for 15 min. at 37°. Protein was determined by the micro-Kjeldahl method.

RESULTS

Magnesium deficiency was clinically evident within 4–5 days after commencement of the low-magnesium purified diet; there were low weight gain and food consumption, difficulty in walking and standing, and occasional magnesium-tetany seizures. Ducks given the low-magnesium diet showed moderate calcium deposition in kidney tubular lumens and in elongated foci in most hearts. These deposits were of lesser magnitude and frequency with higher dietary magnesium.

Table 1 summarizes some factors affecting phosphorylation and the rate of respiration of duck-heart sarcosomes. Regardless of the sucrose concentration or whether or not flasks were supplemented with magnesium, sarcosomes from magnesium-deficient ducks consistently showed a rate of respiration lower than controls. These same factors affected oxidative phosphorylation in duck-heart sarcosomes. Magnesium deficiency was only apparent when P:O values were measured in flasks unsupplemented with Mg²⁺ ions. Control sarcosomes still had a near normal P:O ratio of 2.2, but sarcosomes from magnesium-deficient ducks did not esterify inorganic orthophosphate.

Table 2 shows that sarcosomal adenosine-triphosphatase activity was not affected by the level of dietary magnesium or by the addition of 2,4-dinitrophenol to the flasks. Adenosine-triphosphatase activities were not affected by the absence of magnesium in the incubating medium.

Table 1. *Factors affecting oxidative phosphorylation and the rate of respiration of duck-heart sarcosomes*

Concentration of sucrose is that used in the isolation medium for sarcosomes. q_{O_2} values are μl. of O₂ consumed/mg. of N/hr. α-Oxoglutarate was the substrate. Each figure is the mean result of several experiments.

Mg (mg./100 g. of diet)	Concn. of sucrose (M)	Concn. of Mg in flasks (M)	q_{O_2}	P:O
0	0.88	0.05	106	1.2
192	0.88	0.05	154	1.3
0	0.25	0.05	164	2.4
192	0.25	0.05	280	2.8
0	0.25	—	42	0
192	0.25	—	181	2.2

Table 2. *Adenosine-triphosphatase activity of duck-heart sarcosomes*

Concentration of sucrose is that used in the isolation medium for sarcosomes. Adenosine-triphosphatase activity is μmoles of P liberated/mg. of protein/hr. Each figure is the mean result of several experiments.

Mg (mg./100 g. of diet)	Concn. of sucrose (M)	Concn. of 2,4-dinitro- phenol in flasks (μM)	Adenosine- triphosphatase activity
0	0.88	50	6.9
0	0.88	—	7.4
48	0.88	50	6.7
48	0.88	—	8.0
192	0.88	50	6.2
192	0.88	—	7.4
0	0.25	50	3.8*
0	0.25	—	4.8*
192	0.25	50	4.0*
192	0.25	—	4.3*

* When magnesium was omitted from the incubating medium the adenosine-triphosphatase activity values were not apparently different from the values shown here.

Comparison of the enzymic activities of rat-heart sarcosomes (cf. Table I of Vitale *et al.* 1957*b*), and duck-heart sarcosomes demonstrates the species difference as depicted in the sarcosomes. For all control animals, rat-heart sarcosomes are about twice as active as duck sarcosomes. Heart sarcosomes from magnesium-deficient rats, as compared with controls, show no change in the respiratory rate but do show a lowered P:O ratio. In contrast, heart sarcosomes from magnesium-deficient ducks, as compared with controls, show a lowered respiratory rate with no change in P:O ratio.

DISCUSSION

Table 1 shows that duck-heart sarcosomes have an appreciable rate of respiration when isolated in 0.88M-sucrose, and that their activity increased

when 0.25 M-sucrose was used. These results agree with those of Slater & Cleland (1953), who showed that an increase in tonicity of the extracting medium caused a decrease in activity of the α -oxoglutaric-oxidase system in cat sarcosomes. Positive increases were obtained with 0.25 M-sucrose [cf. the findings of Ziegler, Linnane & Green (1958) that ox-heart sarcosomes isolated in 0.25 M-sucrose appeared swollen and rounded rather than the normal rod-shaped particles that were obtained when prepared in 0.88 M-sucrose].

The low-magnesium diet in no way affected the enzymic content of duck sarcosomes since the particles were able to respire *in vitro* when flasks were supplemented with magnesium. The data also indicate that sarcosomes from magnesium-deficient ducks contain bound magnesium since they have a low but measurable q_{O_2} of 42 when incubated in a magnesium-free medium. The effect of feeding a magnesium-deficient diet to ducks is apparent *in vitro* when sarcosomes are incubated in a magnesium-free medium.

The lowered P:O ratio when 0.88 M-sucrose rather than 0.25 M-sucrose was used for isolating sarcosomes parallels the observations of Slater & Cleland (1953). Their P:O values were 2.7 and 1.4 for cat sarcosomes isolated in 0.25 M- and 0.88 M-sucrose respectively. However, the important result in Table 1 is the zero P:O value for sarcosomes from magnesium-deficient ducks when these sarcosomes were incubated in a magnesium-free medium. Omission of magnesium had no effect on the P:O ratio for control sarcosomes. Apparently, the magnesium present in deficient sarcosomes was either insufficient or in an unsuitable form for coupling oxidation to phosphorylation. Mg^{2+} ions not only preserve mitochondrial structure (Chance & Williams, 1956), but seem to act as a ligand with a soluble component (Linnane, 1958) necessary for respiratory control and active phosphorylation. However, considerable amounts of magnesium are found in membrane fragments that are able to show some oxidative phosphorylation (Lehninger, Wadkins, Cooper, Devlin & Gamble, 1958). The data of Table 1 agree with these findings in that sarcosomes from magnesium-deficient ducks, when incubated in a magnesium-free medium, exhibited a low respiratory rate with no oxidative phosphorylation.

The adenosine-triphosphatase activity of duck sarcosomes (Table 2) differed from other species (Kielley & Bronk, 1957) since no increase in activity resulted when 2,4-dinitrophenol was added to the incubating medium. However, the increased adenosine-triphosphatase activity for sarcosomes isolated in 0.88 M-sucrose as compared with 0.25 M-sucrose parallels Potter & Recknagel's (1951) adenosine-triphosphatase values for rat-liver mito-

chondria. Freshly prepared duck-heart sarcosomes showed adenosine-triphosphatase activity when magnesium or 2,4-dinitrophenol or both were omitted from the incubating medium. Slater (1957) reported that muscle sarcosomes have considerable adenosine-triphosphatase activity further increased by addition of 2,4-dinitrophenol. Holton, Hülsmann, Myers & Slater (1957) found that sarcosomes have a higher adenosine-triphosphatase activity than liver mitochondria in the presence of magnesium and in the absence of 2,4-dinitrophenol. On the other hand, Kielley & Kielley (1951) reported that fresh-muscle mitochondria showed little adenosine-triphosphatase activity even when presented with substrate, magnesium and adenosine triphosphate. Weinbach (1959) also found very little adenosine triphosphatase in fresh mitochondria isolated from liver.

An important difference between rat-heart and duck-heart sarcosomes is the uncoupling effect for sarcosomes from magnesium-deficient animals. Sarcosomes from deficient rats show no change in q_{O_2} as compared with the lowered q_{O_2} for duck sarcosomes. Since all deficient sarcosomes were prepared from 0.88 M-sucrose, swollen duck sarcosomes may lose adenine nucleotides, DPN, TPNH₂, or the cations Mg^{2+} , Na^+ and K^+ (Kielley & Kielley, 1951) more readily than rat sarcosomes to result in the q_{O_2} drop.

The drop in P:O ratio for sarcosomes from magnesium-deficient rats may result from an increased adenosine-triphosphatase activity due to mitochondrial swelling with a consequent shift from 2,4-dinitrophenol- to magnesium-sensitivity.

Some physical or biochemical difference exists between duck-heart and rat-heart sarcosomes.

SUMMARY

1. The respiratory rate and oxidative phosphorylation of duck-heart sarcosomes were increased when sarcosomes were isolated in 0.25 M-sucrose as compared with 0.88 M-sucrose. Conversely, the adenosine-triphosphatase activity was reduced when sarcosomes were isolated in 0.25 M-sucrose.

2. Sarcosomes from ducks given a purified diet low in magnesium exhibited decreased respiration and normal oxidative phosphorylation and adenosine-triphosphatase activity. However, sarcosomes from magnesium-deficient ducks showed no oxidative phosphorylation when magnesium was omitted from the incubating medium.

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Comparative Studies of Waste Nitrogen Metabolism in Amphibia

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Munro (1953) first showed that the fully aquatic South African clawed toad, *Xenopus laevis*, excretes ammonia as its main nitrogenous waste product. More typical frogs, such as *Rana temporaria*, even when given an abundant water supply, produce mainly urea (Munro, 1939). A comparative study of nitrogen excretion in a number of species of frogs (Cragg, Balinsky & Baldwin, 1961) showed that, although the majority are ureotelic, all members of the aquatic family Pipidae, to which *Xenopus* belongs, produce large amounts of ammonia.

In an attempt to explain the ammoniotelism of *Xenopus*, it was decided to investigate the levels of activity of some of the enzymes involved in the formation of each of the two waste products in *X. laevis*, as compared with those of a more typical frog. Urea synthesis in the liver of *Rana esculenta* was first demonstrated by Manderscheid (1933), and shown to resemble the corresponding mammalian process. Brown & Cohen (1960) have demonstrated the presence of all the Krebs urea-cycle enzymes in several species of Amphibia. The activities of all these enzymes increase during

metamorphosis of the tadpole, at the same time as it becomes ureotelic (Dolphin & Frieden, 1955; Brown, Brown & Cohen, 1959).

Very little is known of the mode of ammonia formation in frogs. The work of Balinsky & Baldwin (1961) suggests that in *Xenopus* the process occurs in the kidney. In mammals, it is generally assumed that urinary ammonia is formed mainly by the hydrolysis of glutamine (Van Slyke *et al.* 1943), though other mechanisms have been suggested (Madison & Seldin, 1958), including L-amino acid and glycine oxidases, and glutamic dehydrogenase.

The present paper describes a comparative study of arginase in the liver, and deamination in the kidney of *X. laevis* and *R. temporaria*. A preliminary report of some of the results has already been published (Balinsky, 1960).

MATERIALS AND METHODS

Experimental animals. The species used for the experiments were *X. laevis* and *R. temporaria*. The former, all males, were kept in water in glass aquaria, which were cleaned out frequently. They were fed once a week on chopped liver. The *Rana* specimens used were freshly collected. They were kept for a short time in glass tanks, in half an inch of water. They were not fed, but used for experiments soon after arrival.

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