CCXXXII. MAGNESIUM AND MUSCLE RESPIRATION

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(*Received 19 September 1939*)

SZENT-GYÖRGYI and his school first emphasized the importance of the dicarboxylic acids, succinic, fumaric, malic and oxaloacetic, in the respiration of minced pigeon breast muscle. They indicated that these compounds acted as H carriers between the oxidizable substrates present in their preparations and the cytochrome system. This conception was placed on a firm basis by the work of Stare & Baumann [1936] who showed that these dicarboxylic acids acted in a truly catalytic manner.

In the past two years this theory has been extended by Krebs and his coworkers [Krebs & Johnson, 1937; Krebs & Eggleston, 1938]. They showed that citric acid and its decomposition products, *cis*aconitic acid, *iso*citric acid and α-ketoglutaric acid, exerted a similar effect to the dicarboxylic acids mentioned above; further, they have shown that citric acid is synthesized from oxaloacetic acid and an unknown precursor referred to as triose, though Breusch [1937] has contested this latter claim. On the basis of this work Krebs has postulated the existence, in minced muscle preparations, of a citric acid cycle in which citric acid is first synthesized from oxaloacetic acid and the carbohydrate breakdown product triose, the citric acid so formed is then oxidized to CO_2 and oxaloacetic acid, the net effect being the oxidation of triose. This attractive theory of the oxidation of carbohydrate by muscle is supported by the fact that insulin stimulates the utilization of O_2 by these preparations in the presence of citric acid or its breakdown products [Krebs & Eggleston, 1938] and it also provides an explanation of the CO_2 production.

That boiled muscle or yeast extracts exert a stimulating action on the respiration of muscle has been known for some time, the action being ascribed to the presence of coenzymes and substrates. Greville [1937] showed that fumaric acid, or Mg or coenzyme I, would increase the O_2 consumption of a dispersion of pigeon breast muscle in phosphate buffer of *p*H 7·3; he also demonstrated that boiled yeast extract exerted a stimulating action on his preparation. The present paper is an attempt to determine to what extent the activity of boiled muscle extract is due to the Mg or dicarboxylic acids it contains.

Methods

Material. Pigeon breast muscle was used throughout. The pigeons were killed by decapitation and the pectoral muscles dissected out and cooled in ice. The muscles were then minced in a Latapie mincer which had been cooled in the ice chest. The mince was suspended in *M*/10 phosphate buffer, *p*H 7·4, and 2 ml. were used in each experiment. The boiled muscle extract was made from sheep's heart by the method of Krebs & Eggleston [1938] and the reaction was adjusted to *p*H 7·4 before use.

O_2 consumption was measured by means of the Warburg manometric apparatus. CO_2 was absorbed by 0·3 ml. of 10% NaOH placed in the centre tube

which was fitted with a folded filter paper. All additions were made from the side bulb which, in all cases, contained 1 ml. of fluid, making the total volume in which the tissue was suspended 3 ml. The experiments were carried out in an atmosphere of O_2.

For the estimation of succinic and fumaric acids the ethereal extraction described by Elsden [1938] was used; the succinic acid was estimated by the manometric method of the same author. The estimation of fumaric acid was achieved by a microhydrogenation technique using colloidal Pd as a catalyst, the volume of H_2 absorbed being measured manometrically. The details of this method were worked out by Mr Kenneth Harrison of the Biochemical Laboratory, Cambridge.

Mg was estimated by the oxyquinolate method of Greenberg *et al.* [1935]. Mg was added in the form of the chloride and the strength of the solution was checked by estimation before use. Succinic and fumaric acids were added as the Na salts.

Experimental

The effect of added Mg. Addition of Mg alone to minced muscle caused a slight increase in the amount of O_2 used. The addition of succinate or fumarate alone, as was known from earlier work, produced an increase in O_2 uptake, but in the present experiments the effect was not always catalytic. This discrepancy can be explained in terms of H ion concentration, for Krebs & Eggleston found that the catalytic effect of these substances is more apparent at *p*H 6·8 than at 7·4, the reaction at which these experiments were carried out. The addition of both a dicarboxylic acid and Mg brought about a large increase in the total O_2 consumed. The effect was not simply additive, but was in all cases greater than the sum of the effects of either substance acting alone. The results using fumaric and succinic acids are summarized in Tables I and II respectively. It will be

Table I. *O_2 uptake in presence of fumarate and Mg*

0·37 mg. fumaric acid was added as Na salt. Figures in parentheses represent the wt. of Mg added in mg.

	Dilution of muscle	Time min.	O_2 uptake (μl.) Blank	±Fum.	+Mg	Fum.+Mg	
1	1:10	90	631	990	786	1222	(0·31)
2	1:10	90	571	977	686	1172	(0·31)
3	1:10	90	613	840	691	1171	(0·31)
4	1:10	90	839	1011	850	1532	(0·62)
5	1:10	120	851	1241	1047	1653	(0·31)

Table II. *O_2 uptake in presence of succinate and Mg*

0·38 mg. succinic acid added as Na salt, and 0·31 mg. Mg added as chloride.

Dilution of muscle	Time (min.)	O_2 uptake (μl.) Blank	+Succinate	+Mg	Succinate+Mg
1:10	120	1117	1337	1151	1651
1:10	150	724	970	883	1495
1:20	150	357	440	426	732

seen that where 0·62 mg. Mg was used the effect was certainly not additive; this is also demonstrated in Fig. 1. Table III shows the effect of varying the amount of Mg added, and it will be seen that the magnitude of the effect increases with

increasing amounts of Mg up to 0·62 mg., but that a higher concentration—1·55 mg.—while producing an increase in O_2 uptake over and above the control, is less effective than 0·62 mg. This inhibitory effect was first observed by Greville [1937].

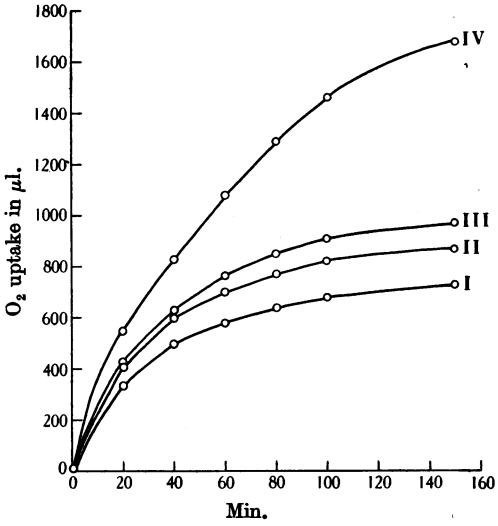

Fig. 1.

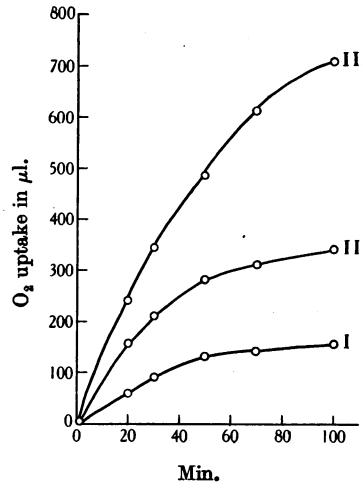

Fig. 2.

Fig. 1. Effects of succinic acid and Mg on the respiration of minced pigeon breast muscle diluted 1:10 with phosphate buffer *p*H 7·4. I, control; II, +0·39 mg. succinic acid; III, +0·62 mg. Mg; IV, 0·39 mg. succinic acid + 0·62 mg. Mg.

Fig. 2. Effect of boiled muscle extract compared with equivalent amounts of Mg and succinic acid on the respiration of minced pigeon breast muscle diluted 1:20 with phosphate buffer *p*H 7·4. I, control; II, +0·1 mg. Mg + 0·22 mg. succinic acid; III, +1 ml. of boiled muscle extract.

Table III. *O_2 uptake with varying concentrations of Mg*

0·37 mg. of fumaric acid added as Na salt present in all cases.

Dilution of muscle	Time min.	O_2 uptake (μl.) Control	0·002 mg. Mg	0·31 mg. Mg	0·62 mg. Mg	1·55 mg. Mg
1:5	85	918	—	1598	2381	2028
1:5	90	824	973	1602	2163	1924
1:10	120	1024	—	1963	1971	1916
1:10	120	1232	—	1443	1816	1635
1:10	90	973	—	1457	1543	1230

The effect of boiled muscle extract. Before use the extract was assayed for Mg, fumaric acid and succinic acid, and for comparison a solution of succinic acid was used equivalent to the total dibasic acids present in 1 ml. of the extract; Mg was added to this solution in an amount corresponding to that in 1 ml. of extract. The results obtained are shown in Fig. 2. It will be seen that the activity of boiled muscle extract exceeds the activity of an equivalent amount of succinic acid and Mg. Boiled muscle extract produced a 350% increase in the O_2 used whereas the succinic acid-Mg mixture caused an increase of 120%. In the experiment quoted 0·1 mg. Mg was used whereas the extract contained only 0·087 mg., a circumstance operating in favour of the mixture.

The effect of iodoacetic acid. In view of the known function of Mg as a cophosphorylase in glycolysing systems the possibility existed that it was playing the same role in the respiration of the muscle preparations described above and, therefore, that part at least of the glycolytic system was functioning here. In order to determine the extent to which this occurred under the aerobic conditions

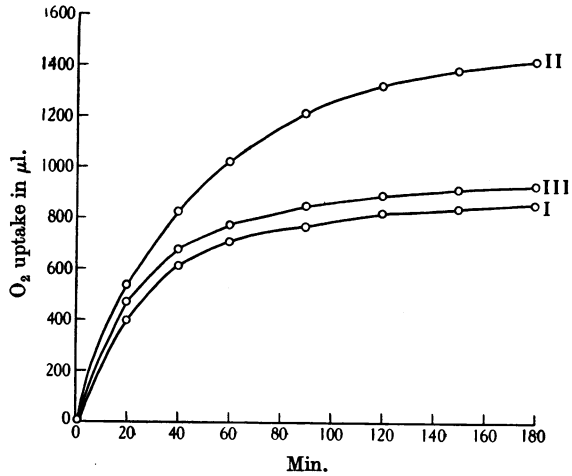

Fig. 3. Effect of iodoacetic acid on the respiration of minced pigeon breast muscle diluted 1:10 with phosphate buffer *p*H 7·4. I, control; II, +0·39 mg. succinic acid +0·62 mg. Mg; III, same as II but with *M*/1000 iodoacetic acid.

of the experiment, iodoacetic acid, in a final concentration of *M*/1000 was added. The results are expressed in Fig. 3. It will be observed that iodoacetic acid gives almost complete inhibition of the succinic acid-Mg effect after a latent period of about 30 min.

Discussion

It is clear from these experiments that the stimulating action of boiled muscle extract on muscle respiration cannot be explained on the basis of its Mg and dicarboxylic acid contents alone, though it is certain that these are essential components of the system. From the work of Greville [1937] it is certain that coenzyme I plays a part, but as no method for the estimation of this substance was available, it was impossible to obtain a quantitative idea of its importance. It is also conceivable that other coenzymes are involved.

The action of the succinic and fumaric acids can be explained on the Szent-Györgyi hypothesis or the citric acid cycle, but the action of Mg is not so clear. Lohmann [1931] showed that Mg formed an essential component of the glycolysing system found in muscle extracts where it acted as a cophosphorylase. Recently, Adler *et al.* [1939] have studied the effect of Mg on the *iso*citric dehydrogenase present in heart muscle and an essential component of the citric acid cycle, and have shown that it is an activator of this enzyme.

On this evidence there are three interpretations of the Mg effect: (*a*) That it is acting solely as a cophosphorylase and therefore its presence stimulates the production of triose, the substance oxidized. (*b*) That it is acting in the capacity of activator to the *iso*citric dehydrogenase. (*c*) That Mg acts in both these ways. In the absence of further evidence this last possibility seems to be the most reasonable.

Krebs [1931] showed that $M/1000$ iodoacetic acid completely inhibited the O_2 uptake in the presence of glucose but not of lactic acid, by slices of rat sarcoma, rat brain cortex and rat testis. Since then iodoacetic acid has been shown to inhibit two mammalian dehydrogenases: the triosephosphate dehydrogenase [Green *et al.* 1937] and the *iso*citric dehydrogenase [Adler *et al.* 1939]. The exact point at which iodoacetic acid acts in the muscle system is not yet clear. It may be inhibiting the triosephosphate dehydrogenase or the *iso*citric dehydrogenase or both. Further work is in progress to attempt to elucidate this point.

SUMMARY

1. Mg, especially in the presence of succinic or fumaric acids, stimulates the respiration of minced pigeon breast muscle.

2. The stimulating action of boiled muscle extract on the respiration of minced pigeon breast muscle cannot be completely accounted for by its Mg and dicarboxylic acid contents.

3. $M/1000$ iodoacetic acid completely inhibits the succinic acid-Mg effect after a latent period of 30 min.

The author wishes to express his thanks to Mr Kenneth Harrison of the Biochemical Laboratory, Cambridge, for the details of the method for estimating fumaric acid, and to the Moray Fund of Edinburgh University which defrayed the expenses of this research.

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