147. THE EFFECT OF ELECTROLYTES ON THE RESPIRATION OF PIGEON BREAST MUSCLE

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It is known from the work of Stare & Baumann [1936], Greville [1937], Elliott & Elliott [1939], Elsdon [1939], and Krebs & Eggleston [1940], that minced animal tissues respire best in a medium containing Na, K, Mg, Cl and PO_4 ions, but no Ca ions. A medium used in this laboratory, in which the above ions are present in approximately optimal concentrations, contains 0.158 *M* Na, 0.00452 *M* K, 0.00113 *M* Mg, 0.122 *M* Cl, and 0.022 *M* PO_4 .

In experiments in which the effect of varying concentrations of Mg on the respiration of muscle tissue was studied, it was found that the optimal concentration of Mg is not constant, but depends on other factors, especially on the concentration of K. Furthermore the optimal concentration of K is not fixed; it is lowered by Mg.

Methods

The manometric experiments were carried out in conical cups. Unless otherwise stated, 3 ml. of a muscle suspension, prepared from 4 g. minced pigeon breast muscle and 45 ml. medium, were measured into the cup and the volume brought to 4 ml. by addition of substrate, electrolytes and water. Fumarate was used as a substrate. The final suspension in the cup always contained 1 part of muscle in 15 parts of medium. The centre cup contained 0.2 ml. 2*N* NaOH. The manometers were filled with O_2 and the O_2 uptake was measured at 40° after an equilibration period of 10 min.

It should be noted that the "phosphate buffer" was prepared from Na_2HPO_4 and HCl, and therefore contained 0.02 *M* NaCl. "Phosphate saline" was the medium described by Krebs & Eggleston [1940]. These authors also give further details regarding the preparation of the suspension and the composition of media.

Effect of potassium

The effect of potassium on the respiration is shown in Table 1 and Fig. 1. K causes a 40–50% increase in respiration, the optimal concentration being 0.04 *M*. This effect of K is especially marked in the later stages of the experiment, as shown in Fig. 1.

Effect of magnesium

The effect of Mg, when added to muscle suspension in phosphate buffer, is shown by Tables 2 and 3.

Mg (0.0125 *M*) activates the respiration of pigeon breast muscle in phosphate buffer 110%. This confirms the results of Greville [1937].

Further experiments show that the optimum Mg concentrations depend on the concentration of other ions in the medium. In a medium containing Na, K, and Cl in "physiological" concentrations, the optimal Mg concentration is

Table 1. *The effect of K on muscle respiration*

1 ml. stock suspension (4 g. tissue in 15 ml. phosphate buffer), 0.1 ml. 0.2 *M* fumarate, 2 ml. salt solution, volume adjusted to 4 ml. with H₂O. For comparison the O₂ uptake of 3 ml. muscle suspension in phosphate saline was measured (cup 7).

Cup	Salt solution added to 1 ml. stock suspension (except cup 7)	Final conc. of K <i>M</i>	μl. O ₂ uptake in 120 min.	% increase due to K
1	2.0 ml. 0.9% NaCl	0	746	0
2	1.5 ml. 0.9% NaCl, 0.5 ml. 1.15% KCl	0.0192	1053	41
3	1.0 ml. 0.9% NaCl, 1.0 ml. 1.15% KCl	0.0385	1141	53
4	0.5 ml. 0.9% NaCl, 1.5 ml. 1.15% KCl	0.0578	1074	44
5	2.0 ml. 1.15% KCl	0.077	1076	44
6	1.9 ml. 0.9% NaCl, 0.08 ml. 1.15% KCl 0.02 ml. 3.84% MgSO ₄	0.0031	1277	—
7	—	—	1865	—

Fig. 1.

Fig. 2.

Fig. 1. The effect of K on muscle respiration. The numbers of the curves correspond with the numbers of the cups in Table 1.

Fig. 2. The effects of Mg and Mn on muscle respiration. The numbers of the curves correspond with the numbers of the cups in Table 2.

Table 2. *The effects of Mg and Mn on muscle respiration*

3 ml. muscle suspension in phosphate buffer (except in cup 5), 0.1 ml. 0.2 *M* fumarate, electrolytes, H₂O up to 4 ml.

Cup	Added electrolyte	Final conc. of added electrolyte <i>M</i>	μl. O ₂ uptake in 120 min.	% increase due to addition
1	0	0	1024	0
2	0.3 ml. 0.01 <i>M</i> MnSO ₄	0.00075	1487	45
3	0.5 ml. 0.1 <i>M</i> MgCl ₂	0.0125	2157	110
4	0.3 ml. 0.01 <i>M</i> MnSO ₄ 0.5 ml. 0.1 <i>M</i> MgCl ₂	0.00075 0.0125	1919	87
5	3 ml. muscle suspension in phosphate saline	—	3247	217

0.0025 *M* (Table 4). If the K concentration is changed to 0.0385 *M*, the optimal Mg concentration is about 0.00125 *M* (Table 5). Addition of Mg to the phosphate saline has no significant effect (Table 6).

Table 3. *Optimal concentration of Mg in phosphate buffer*

3 ml. muscle suspension in phosphate buffer, electrolyte, 0.1 ml. 0.2 *M* fumarate, volume made up to 4 ml. with phosphate buffer.

Cup	Added electrolyte	Final conc. of Mg <i>M</i>	μ l. O ₂ uptake in 120 min.	% increase due to Mg
1	0	0	606	0
2	0.1 ml. 0.1 <i>M</i> MgCl ₂	0.0025	1432	136
3	0.2 ml. 0.1 <i>M</i> MgCl ₂	0.005	1743	188
4	0.4 ml. 0.1 <i>M</i> MgCl ₂	0.01	1932	219
5	0.8 ml. 0.1 <i>M</i> MgCl ₂	0.02	1788	195

Table 4. *Optimal concentration of Mg in a medium containing Na, K, Cl and PO₄ in physiological concentrations*

3 ml. muscle suspension in a solution containing 100 parts 0.9% NaCl, 4 parts 1.15% KCl, 30 parts phosphate buffer. 0.1 ml. 0.2 *M* fumarate, electrolyte, volume made up to 4 ml. with H₂O.

Cup	Added electrolyte	Final conc. of Mg <i>M</i>	μ l. O ₂ uptake in 120 min.	% increase due to added Mg
1	0	0	856	0
2	0.05 ml. 0.1 <i>M</i> MgCl ₂	0.00125	1332	56
3	0.1 ml. 0.1 <i>M</i> MgCl ₂	0.0025	1622	89
4	0.2 ml. 0.1 <i>M</i> MgCl ₂	0.005	1450	69
5	0.4 ml. 0.1 <i>M</i> MgCl ₂	0.01	1112	30

Table 5. *Optimal concentration of Mg in a medium containing 0.0385 *M* K*

1 ml. stock suspension (2 g. minced muscle in 7.5 ml. phosphate buffer). 0.1 ml. 0.2 *M* fumarate, 2 ml. salt solution containing equal parts of 0.9% NaCl and 1.15% KCl, electrolyte, volume made up to 4 ml. with phosphate buffer.

Cup	Added electrolyte	Final conc. of Mg <i>M</i>	μ l. O ₂ uptake in 90 min.	% increase due to Mg
1	0	0	1201	0
2	0.1 ml. 0.025 <i>M</i> MgCl ₂	0.000625	1394	16
3	0.2 ml. 0.025 <i>M</i> MgCl ₂	0.00125	1566	30
4	0.1 ml. 0.1 <i>M</i> MgCl ₂	0.0025	1515	26
5	0.2 ml. 0.1 <i>M</i> MgCl ₂	0.005	1517	26

Table 6. *Effect of Mg added to muscle suspended in phosphate saline*

3 ml. suspension in phosphate saline, 0.1 ml. 0.2 *M* fumarate, electrolyte, volume brought to 4 ml. with H₂O.

Cup	Added electrolyte	Final conc. of Mg <i>M</i>	μ l. O ₂ uptake in 120 min.	% change due to added Mg
1	0	0.00085	1521	0
2	0.05 ml. 0.05 <i>M</i> MgCl ₂	0.00147	1701	+ 12
3	0.1 ml. 0.05 <i>M</i> MgCl ₂	0.0021	1666	+ 9
4	0.2 ml. 0.05 <i>M</i> MgCl ₂	0.00336	1302	- 14.5
5	0.4 ml. 0.05 <i>M</i> MgCl ₂	0.00586	1384	- 9

Effect of manganese

It is known from the work of Negelein (quoted by Warburg & Christian [1936]), Ohlmeyer & Ochoa [1937], and Adler *et al.* [1939], that Mg plays an important part in cell metabolism, which in some cases resembles that of Mn.

As shown by Table 2 and Fig. 2, Mn in low concentrations ($0.75 \times 10^{-3} M$) has an activating effect on the respiration of minced muscle suspended in phosphate buffer. This effect is limited to the first 30 min., and it is not maintained afterwards. Mn thus cannot replace Mg.

Mn has no activating effect when added to muscle suspensions in phosphate saline. In the later periods of the experiment there is an inhibition of the O_2 uptake. The Mn effect on muscle suspensions in phosphate saline does not depend on the substrate added. The following substrates have been tested: fumarate, pyruvate, citrate, α -ketoglutarate, glutamate and boiled muscle extract.

SUMMARY

The O_2 uptake of minced pigeon breast muscle suspended in various saline media was measured.

The optimal K concentration was found to be $0.0385 M$ if the medium contained $0.02 M$ phosphate and $0.0425 M$ NaCl. The optimal K concentration was much lower ($0.0034 M$) when the medium contained $0.02 M$ phosphate, $0.092 M$ NaCl and $0.00085 M$ $MgSO_4$.

The optimal Mg concentration depends on the concentration of K and may vary between 0.00085 and $0.01 M$. Mg and K can partially, but not completely, replace one another.

Mn increases the O_2 uptake of muscle suspended in phosphate buffer, but does not replace Mg.

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REFERENCES

- Adler, Eufler, Günther & Plass (1939). *Biochem. J.* **33**, 1028.
Elliott & Elliott (1939). *J. biol. Chem.* **127**, 457.
Elsden (1939). *Biochem. J.* **33**, 1895.
Greville (1937). *Biochem. J.* **31**, 2274.
Krebs & Eggleston (1940). *Biochem. J.* **34**, 442.
Ohlmeyer & Ochoa (1937). *Biochem. Z.* **293**, 338.
Stare & Baumann (1936). *Proc. roy. Soc. B*, **121**, 338.
Warburg & Christian (1936). *Biochem. Z.* **287**, 294.