REVISTA ESPAÑOLA DE FISIOLOGIA, 27, 155-162. 1971

Oxygen Uptake by Rat Jejunum in Media Devoid of Na^+

R. Jordana* and Carmen Igea

Department of Physiology. University of Navarra
Pamplona (Spain)

(Received on December 22, 1970)

R. JORDANA and C. IGEA. *Oxygen Uptake by Rat Jejunum in Media Devoid of Na^+.* R. esp. Fisiol., 27, 155-162, 1971.

It has been studied the effect of the substitution of Na^+ in the medium on the oxygen uptake of jejunal strips of rat.

In incubation experiments of three hours, the substitution of Na^+ diminished the oxygen uptake increasing in the following order: choline, Tris, Li^+, sucrose or mannitol. The greatest inhibition occured with the non ionic substitutes and with Li^+ among the ionic ones.

After a pre-incubation of one hour in Na^+ free media, the tissue respiration in a medium with Na^+ remained strongly inhibited when the substitute used was mannitol or Li^+. In the other cases the recovery was more or less complete.

These effects are attributed to changes in the intracellular ionic composition induced according to the nature of the substitute for Na^+ in the exterior medium. In the case of Li^+ and mannitol, are also suggested changes in the structure or enzymatic systems of the cell.

The diminished oxygen uptake in media devoid of Na^+ can not be explained solely by the suppression of the Na^+ pump. There are other factors more directly involved in the biochemical respiratory processes that can be altered in a partially or non-reversible manner.

These factors that affect the availability of cellular energy must be taken into consideration when studying the relationship between the active transport of diverse substances and the ionic ambient.

The substitution of Na^+ in the medium of incubation by other cations or non-electrolytes diminishes oxygen uptake by the intestine of rats (1, 6). STAMPA (12), using jejunal strips of rat, observed that the substitution of 50 % or more of Na^+ by Li^+ or mannitol produced an inhibition of oxygen uptake that respectively reached 31 % and 46 % in total substitution of Na^+. The results with intestinal mucosa were very similar (2).

Even the simple pre-incubation of jejunal strips of rat during one hour in a medium in which the Na^+ had been substituted by mannitol was sufficient to observe a diminished oxygen uptake with less utilization of glucose and a similar production of lactate (6), which suggests that an alteration in the oxidative metabolism occurred. An analogous preincubation of intestinal sacs of hamster in mannitol media, notably diminishes their capacity to

* With a grant of «Ministerio de Educacion y Ciencia».

actively transport sugars when these sacs are relocated in a saline medium containing Na^+. This does not occurs when, instead of mannitol, Tris or Choline is used (4). Also, in experiments *in vivo* (9) it has been observed that the inhibition of the active transport of glucose by the removal of Na^+ from the medium is less reversible when mannitol replaced Na^+ than in other substitutions. Both facts could be related to an alteration in the oxidative metabolism of the tissue when Na^+ is substituted by mannitol. On the other hand the incubation of rat liver slices in a medium in which Na^+ was substituted by mannitol diminished the activity of succinate dehydrogenase (5).

It was interesting to find out whether the alterations in the oxidative metabolism observed when Na^+ was substituted by mannitol were due to the absence of Na^+, to the presence of mannitol, or to the shift in the ionic strength of the medium. To this end, experiments were conducted using intestinal strips of rat and substitutes of Na^+ of diverse characteristics regarding ionic condition and cellular penetrability.

Materials and Methods

Wistar rats of both sexes, weighing 180 to 200 g. were utilized. After 24 hours fasting, they were decapitated, a segment of proximal jejunum removed and some strips weighing between 50 to 80 mg obtained. The measurement of oxygen uptake was recorded using the direct method of Warburg (14) in the microrespirometer of Warburg. The apparatus was operated at 37° C, 100 oscillations/minute and an amplitude of 3 cm. The working solutions were derived from Krebs-Ringer solutions prepared as described by UMBREIT *et al.* (13) but buffered with Tris-HCl (7) to avoid, in certain cases, the appearance in the medium of the 20 meq/liter of Na^+ that are present in the phosphate buffer. This solution (KRT/Na^+) was modified substituting NaCl 0.154 M by LiCl 0.154 M, Tris 0.3 M + HCl 0.3 M v/v, sucrose 0.3 M or choline chloride 0.154 M, obtaining, the solutions designated as KRT/Li^+, KRT/Tris, KRT/sucrose and KRT/choline, respectively. These solutions were considered as osmotically equivalent.

By pre-incubation it is meant the placement of the strips in a solution, during one hour, before introducing it in the Warburg apparatus for the measurement of oxygen uptake. Incubation means the placement of the tissue in a Warburg flask, during 3 hours, to record the oxygen uptake.

Results

Oxygen uptake during incubation in media devoid of Na^+.

Table I shows the results obtained for oxygen uptake by rat intestinal strips incubated during 3 hours in a saline medium containing Na^+ (KRT/Na^+) or in media devoid of Na^+ (KRT/Li^+, KRT/Tris, KRT/choline, and KRT/sucrose).

The strips incubated in KRT/Na^+ medium maintained a satisfactory respiratory level during the period of incubation giving values of 3.96, 2.96 and 2.43 μM O_2/100 mg wet weight/hour in the 1st, 2nd, and 3rd hour respectively.

If the Na^+ in the medium is substituted by Li^+ (KRT/Li^+) the uptake of oxygen diminishes, attaining inhibition values of 21 % for the first hour and of 50 % for the third hour. The results are very similar when Na^+ is substituted by Tris (KRT/Tris) although, for the first 60 minutes, the inhibition is somewhat inferior and after three hours it is somewhat higher. If the substitute used is choline, inhibition also occurs although in a lesser degree than that produced by Li^+ or Tris. The highest inhibitions were produced when the substitute used was sucrose, with an inhibition of 30 % for the first hour and an inhibition of 69 % for the third hour. These

Table I. *Effect of the absence of Na^+ substituted by Li^+, sucrose, Tris or choline on oxygen uptake by jejunal strips of rat.*

The strips were maintained during the three hours of incubation in Warburg flasks, the media being KRT/Na^+, KRT/Li^+, KRT/sucrose, KRT/Tris or KRT/choline.

The results are expressed in μM O_2/100 mg wet weight/hour with their standard error. Statistical significance according to the student method $P < 0.001$ in all cases.

The numbers in parenthesis indicate the number of experiments.

Incubation	Time (hours)		
	1	2	3
	O_2 uptake (μM O_2/100 mg w.w.)		
KRT/Na^+ (18)	3.94±0.11	2.92±0.09	2.43±0.13
KRT/Li^+ (18)	3.08±0.13	1.94±0.13	1.22±0.11
KRT/Tris (19)	3.40±0.12	1.96±0.10	1.02±0.04
KRT/Col. (19)	3.53±0.10	2.30±0.05	1.53±0.03
KRT/Sac. (18)	2.74±0.08	1.27±0.07	0.76±0.02
% of inhibition in respect to KRT/Na^+			
KRT/Li^+	21.83	34.46	49.80
KRT/Tris	13.71	33.79	58.03
KRT/Col.	10.41	22.30	37.04
KRT/Sac.	30.46	57.10	68.73

values are of the same order to those obtained by STAMPA (12) for Mannitol.

It must be borne in mind that, of the three ionic substitutes used, Li^+ enters into the cell, choline is actively transported (10) and can be metabolized by the tissue, and Tris can be considered non-penetrating. Mannitol and sucrose are non-penetrating, although sucrose is after undergoing hydrolisis.

OXYGEN UPTAKE IN MEDIA WITH Na^+, AFTER PRE-INCUBATION IN MEDIA DEVOID OF Na^+.

In Table II, are shown the results obtained for oxygen uptake by intestinal strips during their incubation in media containing Na^+, after pre-incubation in different media devoid of Na^+. The controls were pre-incubated in KRT/Na^+ for the same period of time.

Pre-incubation in KRT/Li^+, produces an inhibition of the oxygen uptake during the posterior incubation in KRT/Na^+ that amounts to 32 % for the first hour and to 47 % for the third hour. These values are alike to those described during the permanent incubation in KRT/Li^+. When the pre-incubation is made in KRT/Tris, the inhibition observed is weaker, and inferior to the one obtained by incubation in the same medium. After pre-incubation in KRT/choline, the oxygen uptake is higher than that in the control medium during the first two hours of incubation. The pre-incubation in KRT/sucrose does not give significant differences in the oxygen uptake.

Table II. *Effect of pre-incubation during one hour in the absence of Na^+ substituted by Li^+, sucrose, Tris or choline on the oxygen uptake by jejunal strips of rat.*

The strips were maintained during the 60 minutes of preincubation in media in which Na^+ had been substituted by Li^+, sucrose, Tris or choline.

After this period, measurements of the oxygen uptake of the tissue were taken during three hours in Warburg respirometer in medium with Na^+.

Number of experiments 18 in each group.

Preincubation	Time (hours)		
	1	2	3
	O_2 uptake (μM O_2/100 mg w.w.)		
KRT/Na^+	3.33±0.09	2.45±0.06	1.79±0.09
KRT/Li^+	2.27±0.08	1.32±0.06	0.94±0.04
KRT/Tris	3.26±0.11	2.02±0.05	1.41±0.04
KRT/Col.	3.89±0.11	2.95±0.10	1.85±0.08
KRT/Sac.	3.31±0.13	2.25±0.12	1.61±0.09
Differences in % with respect to preincubated in KRT/Na^+			
KRT/Li^+	31.84 [a]	46.13 [a]	47.49 [a]
KRT/Tris	2.10 [b]	15.92 [a]	21.23 [a]
KRT/Col.	16.81 [a]	20.40 [a]	3.35 [b]
KRT/Sac.	0.60 [b]	8.16 [b]	10.00 [b]

[a] $P < 0.001$; [b] P = no sig.

Discussion

The results demonstrate that the incubation of jejunal strips of rat in ordinary saline medium in which Na^+ has been substituted by Li^+, sucrose, Tris or choline, diminishes the oxygen uptake by the tissue. In the third hour, inhibitions varied between 37 % for choline and 68 % for sucrose. The effect increased in order with the following series: choline < Tris < Li^+ < sucrose (Fig. 1). Sucrose behaves in these experiments like mannitol (12).

The mere pre-incubation in Na^+-free media during one hour, variably alters the posterior oxygen uptake by the tissue in a medium containing Na^+. A clear inhibition occured when the substitute used was Li^+, scarce inhibition when Tris was used and there was no effect with sucrose. If the substitute was choline an initial stimulation in the uptake of oxygen is observed.

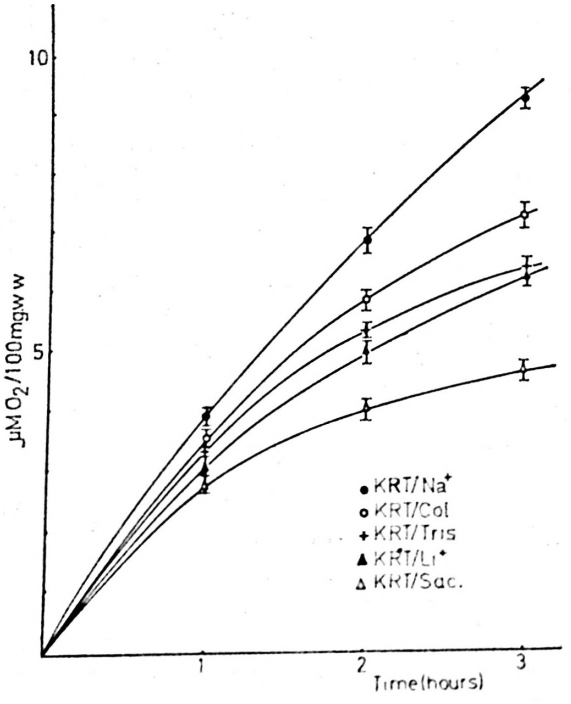

Fig. 1. *Effect of the absence of Na^+ substituted by Li^+, sucrose Tris or choline on oxygen uptake by jejunal strips of rat.*

In the analysis of both types of experiments and their interpretation, it must be borne in mind the different properties of the substitutes employed. The diversity of the results observed with the different Na^+ substitutes revealed that, apart from the simple lack of Na^+, a common situation to all of them, other factors come into play, depending on the nature of the respective substitute that can influence the uptake of oxygen directly or indirectly. This must be so because the mere nonfunction of the Na^+ pump, that had to occur in all the experiments in which Na^+ was absent, can not be sufficient to explain the recorded facts.

The substitution by Li^+ must have been accompanied by a penetration of this cation into the cells, in favor of a concentration gradient, with the exit of other cations, especially K^+, into the medium. The inhibition of the oxygen uptake that was observed during the experiments of incubation can be attributed to the changes in the intracellular ionic composition, which, in some manner, affects the capacity of the tissue for the uptake of oxygen. The pre-incubation in KRT/Li^+ produces the same type of ionic alteration and it is interesting to observe (Fig. 2) that, when the tissue is placed in the control medium containing Na^+, the inhibitory effect is maintained during all of the three hours period of incubation, which goes to proof that the changes induced during the preincubation are not easily reversible. This lack of reversibility suggests that the entrance of a high concentration of Li^+ ions into the cell could render difficult the restoration of the ionic composition of the intracellular fluid when the tissue is placed in the control medium with Na^+, or could perhaps produce irreversible structural or enzymatic alterations related to the oxidation of endogenous substrates. The oxygen uptake in kidney slices is also inhibited if Na^+ is substituted by Li^+ (8). Also, there are numerous observations related to the effects of Li^+ over enzymes,

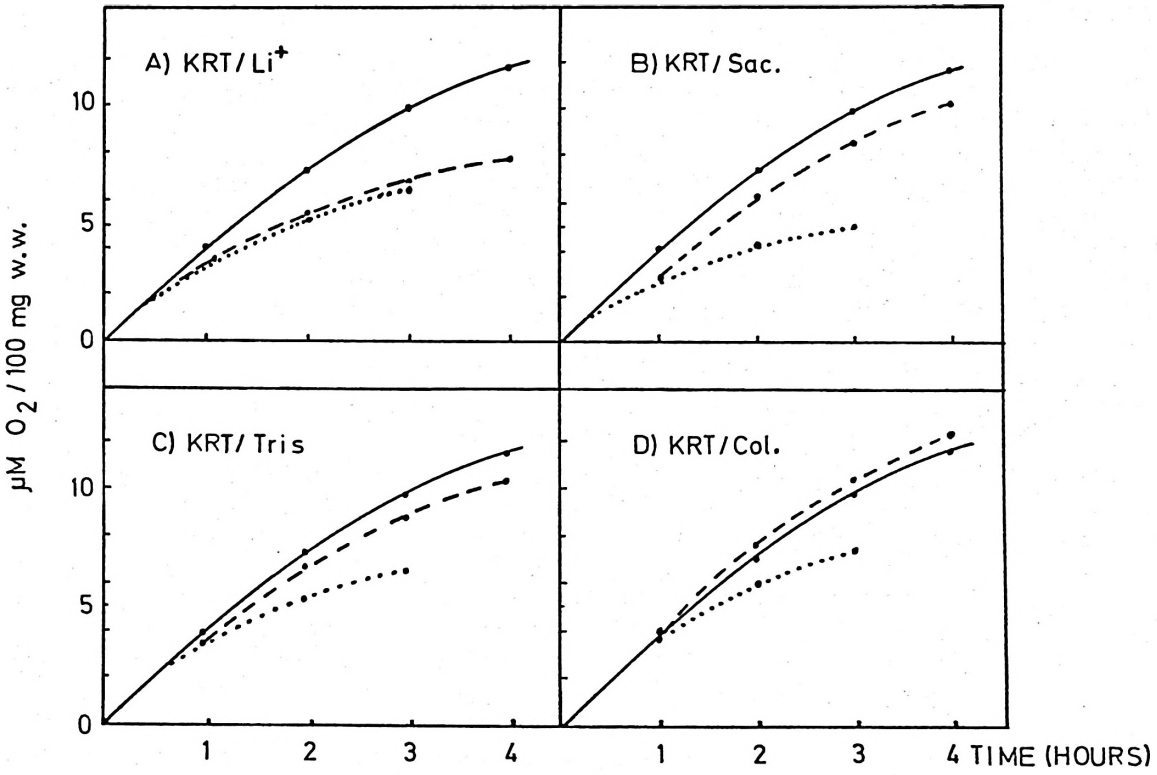

Fig. 2. *Effect of pre-incubation in the absence of Na^+, substituted by Li^+, sucrose, Tris or choline on oxygen uptake recorded posteriorly in the presence of Na^+.*

— Oxygen uptake in KRT/Na^+. --- Oxygen uptake after pre-incubation, compared with that of strips incubated in the same medium. (...) *A*, substitution by Li^+; *B*, by sucrose; *C*, by Tris, and *D*, by choline.

Note in *A* the persistence of the inhibition; in *B*, *C*, and *D*, there is recuperation of the normal oxygen uptake.

diverse metabolic processes, cellular structures, toxic action, etc. (11). This hypothesis is in accordance with the scarce reversibility of the inhibition of the active transport of glucose in the intestine of rat *in vivo* when Na^+ is substituted by Li^+ and replaced again. Even an intestinal washing with LiCl 0.145 M produces inhibition of intestinal absorption of sugar during a certain period (3).

The effects found when Na^+ is substituted by Tris are quite different. In incubation with KRT/Tris there occurs inhibition of the oxygen uptake but it is somewhat inferior to that produced by KRT/Li^+; and after the pre-incubation in KRT/Tris the inhibition is very slight, which implies a good reversibility when Na^+ is restituted to the medium. Tris is a poor or non-penetrating ion (4) which should explain why the intracellular ionic changes are much weaker. The results with KRT/Tris should be fundamentally attributed to the simple absence of Na^+ from the medium.

With KRT/sucrose the oxygen uptake is greatly diminished and in proportions similar to that observed with mannitol (12). In both cases, the substitute is non-ionic and the ionic strength in the exterior medium is very low. This must cause the exit of ions from the cell, producing

a greatly diminished intracellular ionic strength, which in some manner must affect the oxygen uptake. The pre-incubation experiments showed a complete reversibility in the case of KRT/sucrose. When NaCl was restored to the medium, a rapid normalization of the intracellular ionic concentration must have taken place, a process in which the Na^+ pump took part. The reversibility does not occur though, if the pre-incubation was made in KRT/mannitol. This clear difference in the behaviour of sucrose and mannitol, both non ionic and non-penetrating substances, leads to think that mannitol could cause an alteration in the surface of the cell. This will be investigated in the near future.

In the experiments in which choline was the substitute, it was observed that, during incubation, inhibition of the oxygen uptake occurs but to a lesser degree. During pre-incubation the oxygen uptake did not diminished, but, on the contrary, a slight and transitory increase was observed. When NaCl is substituted by choline chloride, the choline penetrates the cell although with some difficulty (4, 10). The exit from the cell of K^+ ions could be as low as when Tris was the substitute. For this reason, the ionic changes in the interior of the cell only minimally affect the oxygen uptake. Another possibility could be that part of the choline that enters the cell will give rise to a high oxygen uptake that would mask the inhibition caused by the substitution of Na^+. When Na^+ is added to the medium, after a pre-incubation in KRT/choline, the intracellular ionic values should return to normal very rapidly.

The metabolizable character of the choline permits us to understand the transitory stimulation in the oxygen uptake produced when choline enters the cell during the pre-incubation period.

From this, we can delucidate that the substitution of Na^+ in the medium, without varying its osmotic properties, brings about a diminished tissue respiratory capacity. This reduction in the respiratory capacity of the tissue varies in accordance with the substitute used and only in part can be explained by the mere absence of Na^+ ions. The effect is greatest when the substitute lacks ionic character (mannitol or sucrose) in which case major changes in the intracellular concentration of diffusible ions must occur. As can be deducted from the pre-incubation experiments, the maintenance of the strips in media devoid of Na^+ affects their respiratory capacity when they are posteriorly placed in the control medium with Na^+, being the reversibility minimal in the case of mannitol and Li^+. Mannitol must affect in some manner the structure and function of the cellular surface. Li^+, because it can penetrate inside the cell, could promote structural or enzymatic alterations that are poorly or non-reversible.

These results are to be considered when one studies the effects produced by the abserce of Na^+ over the active transport of many substances by the intestine because, according to what substitutes are employed to maintain the osmotic pressure of the medium, the respiratory activity of the tissue will be altered to a varying degree and this in turn will have a direct effect over the amount of energy available for the transport process.

References

1. Balasch, J., Stampa, A. and Ponz, F.: *R. esp. Fisiol.*, **21**, 65, 1965.
2. Balasch, J., Stampa, A. and Ponz, F.: *Actas IX R. Nal. Soc. esp. C. Fisiol.*, 1965.
3. Bello, J., Fernández-Otero, P., Durán, E. and Larrai de, J.: *R. esp. Fisiol.*, **19**, 63, 1963.
4. Bosackova, J. and Crane, R. K.: *Biochim. Biophys. Acta*, **102**, 423, 1965.
5. Jordana, R. and Contreras, J.: *R. esp. Fisiol.*, **26**, 175, 1970.
6. Jordana, R. and Ponz, F.: *R. esp. Fisiol.*, **25**, 129, 1969.

7. MICHAELIS, L.: *J. Biol. Chem.*, **87**, 33, 1930.
8. MUDGE, G. H.: *Amer. J. Physiol.*, **167**, 206, 1951.
9. PONZ, F. and LLUCH, M.: *R. esp. Fisiol.*, **20**, 179, 1964.
10. SANFORD, P. A. and SMYTH, D. H.: *J. Physiol.*, **205**, 160, 1969.
11. SCHOU, M.: *Pharmacol. Rev.*, **9**, 17, 1957.
12. STAMPA, A. and PONZ, F.: *R. esp. Fisiol.*, **25**, 69, 1969.
13. UMBREIT, N. W., BURRIS, R. H. and STAUFFER, J. F.: Manomethic Methods. Burgers Publ. Co. Minneapolis, 1951.
14. WARBURG, O.: *Biochem. Z.*, **142**, 317, 1924.