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Oxygen Uptake by Liver Strips in the Absence of Na^+

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The incubation of liver slices during three hours in media devoid of Na^+, substituted to maintain osmolarity by mannitol, sucrose, glucose, Li^+, K^+ or Tris, produces inhibition of the oxygen uptake that is greatest with mannitol (66 % during the third hour), somewhat less with sucrose or glucose and inferior, and very similar, with any of the three other replacements.

After one hour of incubation in different Na^+-free media, the succinate dehydrogenase activity diminishes if the medium used contained mannitol, sucrose or glucose, all with a very low ionic strength.

If after pre-incubation of the slices in Na^+-free medium, they are incubated in control medium with Na^+, the oxygen uptake returns almost completely to normal when the substitute used in the pre-incubation was glucose, sucrose, Li^+ or Tris. In the case of mannitol or K^+ the inhibition of the oxygen uptake was irreversible.

The ionic composition of the external medium affects the cellular metabolism, besides by the absence of Na^+, a factor common to all media employed, by the changes that can be induced in the intracellular medium, in enzyme systems or in cellular structures, according to the nature of the substance which has been choosen for the Na^+ replacement. With mannitol, the greatest and more irreversible inhibition of oxygen uptake and of succinate dehydrogenase activity are accomplished, which must be a consequence of some peculiar action of mannitol.

The suppression of Na^+ from the medium, substituted to maintain osmolarity by other cations or non-electrolytes, causes a diminished oxygen uptake in differtnt tissues (1-5, 8, 11, 14, 15). Frequently, this effect has been related to the cease of the activity of the Na^+-pump and it has been utilized to measure the energetic requirements of this last process (5, 15). Nevertheless, some observations lead to think that the absence of Na^+ could provoke alterations in the cellular respiration not explainable by this cause and suggests structural or functional alterations in the respiratory systems (1, 2, 3, 11). In intestinal mucosa (1, 9) and in the wall of the jejunum of rat (1, 2, 11) there is a clear inhibition in the oxygen uptake when Na^+

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is substituted by mannitol or Li^+. If after a period of incubation in the absence of Na^+, this ion is added to the medium, the oxygen uptake does not normalizes easily (11). The preincubation of jejunal strips in a medium with complet replacement of Na^+ by mannitol, diminshes the subsequent respiration of the tissue in a medium with Na^+, with a diminished uptake of external glucose and an increase in aerobic glycolysis (2). In a medium devoid of Na^+ (with mannitol) the strips practically stop respiration after three to four hours, while the controls with Na^+ continue their oxygen uptake during a much longer period (11). The effects of incubation and pre-incubation in different media devoid of Na^+ over the oxygen uptake in jejunal strips differ according to the nature of the substances used to maintain the osmolarity of the medium (3).

It was interesting to study in other tissues the influence of the nature of the replacement of Na^+ over the oxygen uptake. In the present work the influence of incubation and pre-incubation in media which Na^+ has been substituted by Li^+, K^+, Tris, choline, mannitol, sucrose and glucose over the oxygen uptake by rat liver slices has been investigated.

Materials and Methods

Wistar rats from both sexes of 120 to 180 g. after 48 hours fasting were sacrificed by decapitation, the liver removed, and from its greater lobe two halves were obtained. One was introduced in the control medium and the other in medium devoid of Na^+, both at 0,4° C, and washed to remove as much blood as possible. From each half, slices of less than 0.5 mm thickness were prepared and washed again in cold medium with or without Na^+. The wet weight of the slices was determined, weiching between 65-85 mg and then were placed in Warburg flasks containing 2.5 ml of medium and 0.2 ml of 10 % KOH in the central well. These procedures took from 15 to 20 minutes.

The meassure of the oxygen uptake was done by the direct method of Warburg (13) with the Braun-Melsungen Model SL-85 apparatus, at 37° C, 100 oscillations/minute and 3 cm amplitude. The equilibration time was 10 minutes and it was operated in oxygen atmosphere.

The control medium, with Na^+, has been the Krebs-Ringer solution, prepared as described by UMBREIT *et al.* (12), but buffered with Tris-HCl (2) instead of phosphates (11). The media without Na^+ had the same composition of the control except that NaCl 0.154 M was substituted by LiCl, KCl, Tris (Tris hydroxymethylaminemethane) chloride, always at 0.154 M, or by mannitol, sucrose or glucose 0.3 M. All these media are considered osmotically equivalent and are designated as KRT/Na^+ (control) or KRT/Li^+, KRT/K^+, KRT/Tris, KRT/choline, KRT/mannitol, KRT/sucrose and KRT/glucose.

In the experiments of incubation, the slices were maintained in the Warburg flasks, suspended in the corresponding medium during three hours, and the oxygen uptake recorded for each one of the 3 hours.

In the experiments of *pre-incubation*, the slices were placed in erlenmeyer flasks containing 3.5 ml of the desired medium per 100 ml w. w. of tissue, at 37° C, with gentle shaking and constant bubbling of oxygen during one hour. At the end of this period they were rapidly chilled, the slices washed in cold KRT/Na^+ medium, their wet weight again determined, and then introduced in the Warburg flasks that always contained the control medium (KRT/Na^+). The oxygen uptake was measured during the following three hours.

The mean values of the oxygen uptake are given in micromoles of oxygen/100 mg of wet-weight of tissue, with their standard error and the number of individual experiments.

The activity of succinate dehydrogenase was determined in homogenates prepared from slices maintained during one hour in the control medium or in media devoid of Na^+. The homogenation was of the Potter-Elvhejaem type, with a 0.25 M sucrose volume eight times the wet weight of the tissue, during a period not longer than three minutes and in cold. The determination was made by the succinate manometric method (10) expressing the results in micromoles of oxygen per mg of protein and per minute. The protein was determined according to LOWRY (6).

Results

1. OXYGEN UPTAKE DURING THE INCUBATION IN MEDIA DEVOID OF Na^+. The oxygen uptake of liver slices incubated during 3 hours in control medium (KRT/Na^+) and in a series of Na^+-free media is shown in Table I.

In KRT/Na^+, without external substrate, the slices take up O_2 with an acceptable intensity during the three hour period, ranging from 4.98 to 4.12 μM of O_2/100 mg/hour. These values are somewhat higher than those of intestinal strips (3) and the respiration is maintained better throughout the whole period.

If the slices are incubated in a medium devoid of Na^+ the oxygen uptake is always lower (Fig. 1). However, the importance of this diminution is dependent upon the nature agent used for the Na^+ replacement. Greater inhibitions are obtained with non-electrolyte substances (mannitol, sucrose or glucose) than with the others (Li^+, K^+ or Tris). Also, the effect over the oxygen uptake becomes more apparent in all cases as time passes by. The highest inhibitions are found with replacement of Na^+ by mannitol and ranges between 35 % during the first hour of incubation to 66 % during the third hour, with a clear tendency for the oxygen uptake to end the number of experiments of each

Table I. *Oxygen uptake during incubation in Na^+-free media.*

Rat's liver slices were incubated during three hours in different media in which Na^+ was substituted by other cations or non-electrolytes. The oxygen uptake is given in μM O_2/100 mg wet weight/hour. Together with the mean values, the standard error and the number of experiments ( ) are given. All of the inhibitions are significant with $P < 0.001$.

Incubation	Time (hours)		
	1	2	3
	O_2 Uptake (μM O_2/100 mg w. w.)		
KRT/Na^+	4.98±0.063 (103)	4.47±0.06 (102)	4.12±0.07 (99)
KRT/Mannitol	3.22±0.14 (17)	2.00±0.14 (17)	1.38±0.16 (14)
KRT/Sucrose	3.29±0.15 (18)	2.32±0.09 (18)	1.65±0.15 (18)
KRT/Glucose	3.62±0.13 (22)	2.91±0.07 (22)	2.30±0.06 (22)
KRT/Li^+	3.88±0.12 (29)	3.32±0.08 (29)	2.67±0.11 (29)
KRT/K^+	4.12±0.13 (17)	3.25±0.15 (17)	2.69±0.13 (17)
KRT/Tris	4.14±0.09 (23)	3.18±0.07 (23)	2.54±0.09 (23)
	% of inhibition in respect to KRT/Na^+		
Mannitol	35.34	55.25	66.50
Sucrose	33.93	48.09	59.95
Glucose	27.30	34.89	44.17
Li^+	22.08	25.72	35.19
K^+	17.26	27.29	34.70
Tris	16.86	28.85	38.34

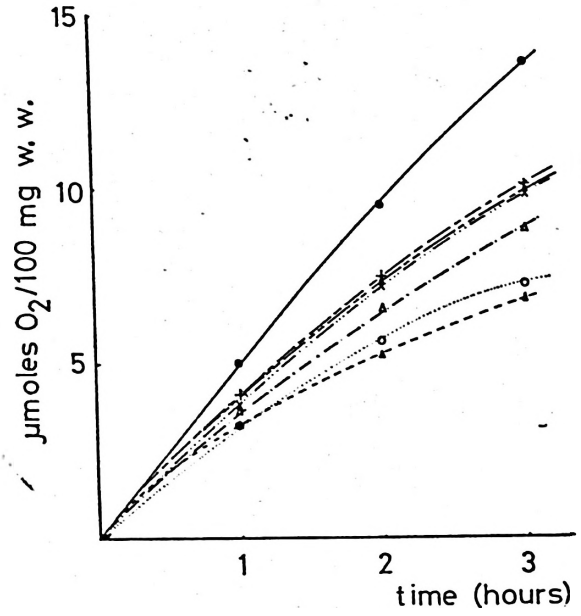

Fig. 1. *Effect of the absence of Na^+ substituted by mannitol (▲), saccharose (○), glucose (△), Li^+ (×), K^+ (+) or Tris (●) on oxygen uptake by rat's liver slices.*

type is highly enough and the dispersion sufficiently reduced to assure the significance of all the inhibitions found.

With Li^+, K^+ or Tris the inhibitions are very similar. The substitution of Na^+ by any of these three ions seems to be equivalent with respect to the diminution of the oxygen uptake, being less noxious than the substitution by non-electrolytes (mannitol, sucrose or glucose). This suggest that when the Na^+-free medium posseses a very low ionic strength, the inhibition of the oxygen uptake is stronger than when it remains equal.

Other experiments were made with KRT/choline. In this case, even though no Na^+ was present, the oxygen uptake does not diminishes; instead it increases to 45 % during the 1st hour and 19 % during the 3rd. It was thought that the choline could be easily oxidized by the tissue, masking the effect of the absence of Na^+. In effect, the addition of 10 mM choline to the KRT/Na^+ medium of incubation, with a slight increase in osmolarity produces an increase in the oxygen uptake that amounts to 31 % during the first hour and to 22 % during the third. If we bear in mind that in KRT/choline medium choline is at a concentration of 154 mM, and that, in spite of this, the oxygen uptake diminishes as the experiment evolves, it seems reasonable to deduce that the substitution of Na^+ by choline progressively inhibits the respiratory capacity of the tissue. The fact that choline is a metabolizable substrate explains why a reduction in the oxygen uptake does not occurs, but instead a small increase occurs with respect to the one that would have been produced in a Na^+ medium to which choline has been added. The excess of substrate in the external medium excludes the possibility of blaming the diminished oxygen uptake throughout the experiment to a fall in the choline concentration.

Mannitol and sucrose can be considered to be not metabolized by the liver under experimental conditions and with both, similar inhibitions are produced. Glucose can be utilized by the tissue and its presence in the external medium provokes an increase in the oxygen uptake. When the slices are in KRT/glucose medium, with 0.3 M glucose, the values for the oxygen uptake are greater than when the medium of incubation is KRT/mannitol or KRT/sucrose. This apparent lower inhibition found when Na^+ is substituted by glucose, with respect to the one obtained with mannitol or sucrose, can be explained if we consider that in the case of glucose the effects is partially masked by the utilization of oxygen required for the oxidation of the fraction of glucose that has entered the cells under the strong concentration gradient.

2. OXYGEN UPTAKE AFTER THE PRE-INCUBATION IN MEDIUM DEVOID OF Na^+. In these experiments the slices were maintained during one hour in different Na^+-

Table II. *Oxygen uptake after pre-incubation in Na^+-free media.*

Rat liver slices were pre-incubated during one hour in different media free of Na^+, substituted by other cations or non-electrolytes. Afterwards, the oxygen uptake in medium KRT/Na^+ was measured during three hours, expressing the results in μM O_2/100 mg wet-weight/hour. The mean values with the standard error and the number of experiments ( ) are given.

Preincubation	Time (hours) 1	2	3
KRT/Na^+	3.46±0.091 (111)	3.09±0.06 (111)	2.70±0.06 (105)
KRT/Mannitol	2.41±0.18 (10)	1.83±0.16 (10)	1.67±0.16 (10)
KRT/Sucrose	3.19±0.09 (18)	2.69±0.11 (18)	2.13±0.07 (18)
KRT/Glucose	3.27±0.09 (21)	2.79±0.07 (21)	2.20±0.07 (21)
KRT/Li^+	3.10±0.158 (20)	2.89±0.171 (20)	2.65±0.18 (20)
KRT/K^+	2.59±0.12 (17)	2.19±0.14 (17)	2.04±0.14 (17)
KRT/Tris	3.13±0.18 (21)	2.60±0.14 (21)	2.33±0.13 (21)
	Differences in % with respect to Pre-incubated in KRT/Na^+		
Mannitol	30.34 [a]	41.10 [a]	38.14 [a]
Sucrose	7.80 [b]	12,94 [a]	21.11 [a]
Glucose	5.49 [b]	9.70 [a]	18.51 [a]
Li^+	10.40 [b]	6.47 [b]	1.85 [b]
K^+	25.14 [a]	29.12 [a]	24.44 [a]
Tris	9.53 [b]	15.85 [b]	13.70 [b]

a) $P < 0.001$; b) P = no signif.

free media and after this period the oxygen uptake during three hours of incubation in Na^+-medium was measured. A number of slices were pre-incubated in KRT/Na^+ medium and served as control. In this way, it could be seen whether the maintenance of the tissue in a medium devoid of Na^+ affected the oxygen uptake in a reversible or not reversible way.

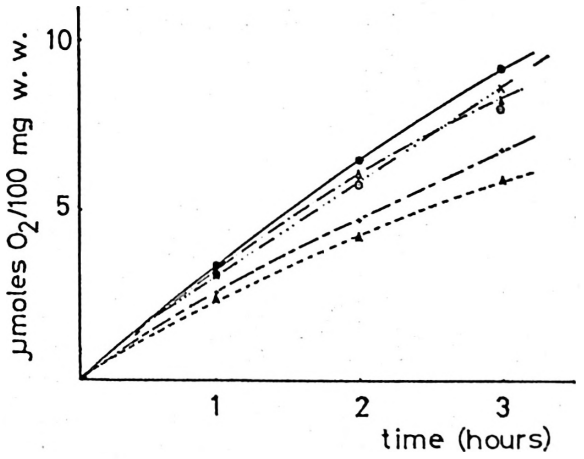

Fig. 2. *Effect of pre-incubation in the absence of Na^+ on the oxygen uptake by rat's liver slices.*

Na^+ (●); Man (▲); Sac (○); Glu △); Li^+ (×); K^+ (+) and Tris (·).

A first observation (Table II) is that the controls in these experiments, pre-incubated during one hour in medium with Na^+, present an oxygen uptake inferior to that of the not pre-incubated controls (Table I), which must be a consequence more to the manipulation to which they were submitted than to the time elapsed from the preparation of the slices.

As can be observed in Table II and Fig. 2, the pre-incubation results in media devoid of Na^+ are quite different according to the nature of the medium. Pre-incubation in mannitol medium permanently inhibits 30 % to 40 % of the subsequent oxygen uptake in medium with Na^+, at least during the three hours of the experiment. In the case of Na^+-replacement by sucrose, glucose, Li^+ or Tris, the oxygen uptake when Na^+ is present in the

medium, returns to practically the same level as that of the controls.

By pre-incubation in KRT/K^+ medium the effect is similar to that found by pre-incubation in mannitol, the oxygen uptake remaining inhibited when the slices are transferred to the medium with Na^+ in similar proportion to that observed by permanent incubation in KRT/K^+.

It can be deducted, therefore, that during the pre-incubation of liver slices in medium devoid of Na^+, substituted by mannitol or K^+, persistent alterations in the metabolic capacity of the tissue are produced and these alterations are maintained when Na^+ is present again in the medium. With other replacements the reversibility is almost complete. With glucose and sucrose a progressive fall in the oxygen uptake can be observed during the period of incubation, proportionally greater than the one observed in the controls, reaching an inhibition value of 20 % during the third hour.

3. EFFECT OF INCUBATION IN MEDIUM DEVOID OF Na^+ OVER THE SUCCINATE DEHYDROGENASE ACTIVITY. After maintaining the slices during one hour in media with or without Na^+, the succinate dehydrogenase activity was assayed in the corresponding homogenates. It is observed in Table III that the oxidation of succinate is inhibited from 15 % to 20 % when for Na^+ substitution mannitol, sucrose or glucose were used, all of them non-electrolytes. With substitution by Li^+, K^+ or Tris, there is no appreciable effect.

Table III. *Succinate dehydrogenase activity after incubation in Na^+-free media.*
Rat liver slices were incubated during one hour in different media devoid of Na^+, substituted by other cations or non-electrolytes. Afterwards they were homogenates in 0.25 M sucrose and the enzyme activity, expressed in μM O_2/mg of protein/minute, was determined. Each mean value is accompained by the standard error and the number of experiments. Only the differences with mannitol, sucrose and glucose are significant ($P < 0.001$).

Incubation	Activity	N.º Exp.	Differences %
Na^+	0.0750 ± 0.00076	191	
Mannitol	0.0595 ± 0.0013	34	— 20.66
Sucrose	0.0636 ± 0.00084	54	— 15.20
Glucose	0.0631 ± 0.0011	35	— 15.86
Li^+	0.0782 ± 0.0013	44	+ 4.26
K^+	0.0789 ± 0.0017	31	+ 5.20
Tris	0.0760 ± 0.00107	48	+ 2.40

Discussion

The incubation of liver slices in medium devoid of Na^+ produces, with all the substances employed to maintain the osmolarity of the medium, inhibitions in the oxygen uptake. That the degree of the inhibition is dependent upon the nature of the substitution shows that, aside from the simple absence of Na^+, a common situation to all cases, other factors are of importance. The greatest inhibitions are reached with non-electrolytes, precisely the ones that produce inhibitions in the activity of the succinate dehydrogenase system. In these cases, the ionic strength of the external medium is very low and it is probably the cause of the greater inhibition of the oxygen uptake and the less succinate dehydrogenase activity. A fact to bear in mind is that the substitution by mannitol is particularly effective in both actions. The fact that there is no significant difference between the inhibition produced by the incubation in Li^+, K^+ or in Tris, all having different characteristics, speaks in favour of, in liver slices, the absence of Na^+, substituted by other cations, is accompanied by a diminished oxygen uptake not withstanding the nature of the respective cation.

The results of the pre-incubation experiments in Na^+-free media offer a particular interest. Of the three non-electrolytes used, mannitol irreversibly affects the tissue in regard to its capacity of oxygen uptake. With the other two, glucose or sucrose, when Na^+ is restituted to the me-

dium, the respiration returns to normal but after a while it becomes inhibited reaching a value of 20 % during the third hour. With Li^+ the reversibility is initially good and becomes complete after some time. The same effect occurs with Tris but the reversibility is not complete. And with K^+ as in the case of mannitol, there is a persistent perturbation of the respiratory activity.

The interpretation of these results appears to be somewhat complex. The substitution of Na^+ by non-electrolytes supposes that the external medium has a weak ionic strength that, among other possible effects, can determine profound changes in the intracellular ionic composition. This must alter the functioning of the respiratory enzyme systems, at least in the activity of succinate dehydrogenase, or the access to them by their corresponding substrates, resulting in a diminished oxygen uptake. Returning to the medium with Na^+ after a pre-incubation in sucrose or glucose, there is a tendency to rapidly restore the intracellular ionic balance, and the oxygen uptake does not significantly differ from the controls. Nevertheless, it is possible that some alteration continues its course and provokes inhibitions, specially during the third hour. With mannitol, besides the ionic changes, there are others that irreversibly affect the tissue. A similar effect characteristic of mannitol has been observed in intestinal strips (3).

In the case of replacement of Na^+ by other cations, changes in the intracellular ionic medium must also happen, although with inferior variations because the ionic strength in the external medium is the same as in the control medium with Na^+. The diminished oxygen uptake could perhaps be attributed simply to the absence of Na^+, by ceasing the activity of the Na^+-pump. With Li^+ or Tris, when returning to the medium with Na^+, the oxygen uptake becomes almost normal which represents a clear difference with respect to what occurs in analogous experiments in intestinal strips, where Li^+ altered the cellular respiration with no reversibility of the effect in 3 hours (3). The pre-incubation in KRT/K^+ produces lasting consequences as occured in the pre-incubation in KRT/mannitol, although the mechanism by which the irreversible inhibition of the oxygen uptake occurs, must be different in each case; with K^+, for example, the activity of succinate dehydrogenase is not inhibited.

As a whole, the results reported in this and in a previous paper concerning intestinal strips (3) show that the cellular metabolism is dependent not only upon the presence of Na^+ in the external medium but also upon the nature of the replacement adopted to maintain the osmolarity of the medium, with differences that can be attributed to various changes induced in the ionic composition of the intracellular medium, that in some way affects the corresponding enzyme systems. In certain cases the metabolic alterations do not disappear when returning to the medium with Na^+, and this occurs in liver slices with mannitol and K^+.

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