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Effects of the Pre-incubation in a Na^+-free Medium on the O_2 Uptake and Glucose Utilization by the Intestine *

by

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It has been observed in tissues other than intestine that the substitution of Li^+ and some non-electrolite compounds for Na^+ is followed by a reduction in O_2 uptake (13, 23). Similar results have been obtained in our laboratory where rat intestinal mucosa and strips of intestine wall have been used (2). The substitution of isosmotic concentrations of Li^+ or mannitol for Na^+ caused a reduction in the O_2 uptake, which was partially dependent on the amount substituted. The replacement of Na^+ by a value up to 50 % did not produce any effect on the O_2 uptake. Further increases in the amount of Na^+ substituted, reduced the O_2 uptake progressively. Complete replacement of Na^+ by Li^+ and mannitol decreased the O_2 uptake in the intestine wall preparation by 30 and 45 %, and in the intestinal mucosa preparation by 52 and 63 %, respectively. Thus, mannitol was consistently more potent inhibitor than Li^+. This effect could be partially explained by the fact that mannitol strongly modifies the ionic strength of the medium. Furthermore, mannitol as opposed to Li^+, scarcely penetrates inside the cell (10).

The values of O_2 uptake of the intestinal mucosa were higher than those observed for the jejunal strips (7, 8). This is not surprising since the mucosa has a higher respiratory activity. In fact, most of the respiratory activity of the jejunal strips is accounted for by the mucosal activity.

It seems evident that the lack of Na^+ affects the cell metabolism to a certain extent, since tissue respiration under these conditions terminates earlier than in the presence of Na^+, even when the osmotic equilibrium is maintained (20). If Na^+ is subsequently restored, O_2 uptake remains below normal values.

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It is known that the presence in the medium of sugars that can be metabolized, such as glucose and fructose, enhances the O_2 uptake (1, 7, 20). If the lack of Na^+ would disturb selectively the active transport processes, the O_2 uptake would be affected only when glucose was incorporated into the system, since glucose transport, and not fructose transport, is Na^+-dependent. However, O_2 uptake in the presence of fructose is also affected by the absence of Na^+ (20).

The present investigation is aimed to study the effects that one hour preincubation of intestine in a Na^+-free medium induces on the subsequent metabolism of the tissue, when Na^+ is reincorporated into the system.

Materials and Methods

White wistar rats of both sexes, weighing 140-200 g, were used for these experiments. The suspensión medium was the Krebs-Ringer solution, as described by UMBREIT et al (21) but with Tris-HCl buffer (pH: 7.4) in order to eliminate 20 mEq/l of Na^+ that would be brought about by the phosphate buffer solution (15).

The animals were killed with a blow on the neck after 24 hr fasting. A segment of proximal jejunum, 20-25 cm. long, was removed and carefully rinsed with distilled water, inside and outside. The segment was divided into strips, 50-70 mg weight, some of which were suspended in a Krebs-Ringer-Tris (KRT/Na) solution, and the others in a Krebs-Ringer-Tris solution where mannitol (0.3 M) was substituting for NaCl (0.154 M) (KRT/Man). Thus, isosmotic solutions were used throughout.

After keeping both groups of strips in their respective solutions at 37° C for 60 min (preincubation time), the strips were rinsed several times with KRT/Na and transferred into the Warburg flasks containing 2.5 ml of KRT/Na, with or withouth glucose, 2.77 mM.

O_2 uptake was measured by Warburg's direct method (22), in an O_2 atmosphere; carbon dioxide was fixed with 10 % KOH solution; the shaking was maintained at 100 oscillations/min, 3 cm amplitude. Measurements were performed every 60 min, for 7 hr. The results were expressed in μM O_2/100 mg wet weight. The dry weight of tissue, as determined in a good deal of strips, was $17 \pm 0{,}5$ % of the wet weight.

Glucose and lactate present in the medium were measured at the end of each experimental period. The medium and the tissue were transferred into a Potter homogenizer; the proteins were precipitated from the homogenate (9), and aliquots of the supernatant were taken for determinations. The glucose was evaluated by a colorimetric method with glucose-oxidase (12), and the lactate by the Barker and SUMMERSON method (3).

Results

1. *Experiments performed in a glucose-free medium.*

The O_2 uptake was measured for periods of 1 to 5 hr, and the lactate concentration at 1, 3 and 5 hr of incubation. The results are shown in Table I and Figure 1. The O_2 uptake of the control strips increased regularly during the 5 hr period. The slowing down of the uptake during the experiment was due to the preincubation period in an air atmosphere, at 37° C for 60 min (11, 20). On the other hand, when the jejunum strips were preincubated in a Na^+-free medium (KRT/Man), the O_2 uptake was lower than that observed in control preparations. A 20 % inhibition was found at the first hour, and 40 % at the fifth hour. It is interesting to point out that the inhibitory effect, induced by the preincubation in a Na^+-free medium, was becoming more evident as the incubation in the presence of Na^+ was proceeding.

TABLE I

Effects of the preincubation without sodium (mannitol) on the O_2 uptake and the lactate production by strips of rat jejunum

Preincubation of the strips in Krebs-Ringer-Tris/mannitol medium for 60 minutes. The control strips were suspended in Krebs-Ringer-Tris/Na^+ medium during the preincubation period. After preincubation the O_2 uptake and the production of lactate were measured in Warburg flask in Krebs-Ringer-Tris/Na^+ medium without glucose. The mean values ($\bar{x}$) are expresed in μM/100mg wet weight. ϵ = Standard error of the mean; N.° = number of tests. Statistics according to Student's method

		Time (hours)				
		1	2	3	4	5
		O_2 uptake (μM/100 mg w.w.)				
Control	$\bar{x}$	3.68	6.55	8.70	10.72	12.25
	ϵ	0.07	0.14	0.21	0.36	0.45
	N.°	138	94	81	53	48
Preincubated without Na^+ (with mannitol)	$\bar{x}$	2.97	4.59	5.62	6.85	7.42
	ϵ	0.09	0.22	0.28	0.47	0.70
	N.°	37	25	22	17	14
Significance		$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$
Differences %		19.29	29.92	35.40	36.10	39.42
		Final Lactate (μM/100 mg w.w.)				
Control	$\bar{x}$	1.09		1.01		1.18
	ϵ	0.08		0.05		0.04
	N.°	39		30		42
Preincubated without Na^+ (with mannitol)	$\bar{x}$	1.27		0.84		1.18
	ϵ	0.19		0.14		0.19
	N.°	12		9		14
Significance		no sig.		no sig.		no sig.
Differences %		16		16		—

Total lactate in the medium was slightly increased (16 %) after 1 hr, and remained unchanged after 3 and 5 hr.

2. *Experiments performed in a glucose medium.*

The solution used in the Warburg flasks was KRT/Na, to which glucose, 2.77 mM, was added. O_2 uptake was measured at 60 min intervals for 7 hr, and glucose and lactate concentrations at 3 and 7 hr during Warburg incubation. The results are shown in Table II and Fig. 2 and 3. In the presence of glucose, the inhibitory effect of the preincubation on the O_2 uptake was less evident than that observed in the absence of glucose; a 20 % inhibition was attained at the second hour, and remained practically unchanged for the rest of the experiment.

After 3 hr of Warburg incubation, glucose utilization was clearly reduced in the strips preincubated in a Na^+-free medium; no differences, however, between both preparations were observed after 7 hr. Lactate production after 3 hr was similar for the two preparations, whereas, after 7 hr, significantly more lactate was pro-

duced in the strips preincubated in a Na^+-free medium.

It must be pointed out that, between the third and the seventh hour, more glucose was utilized in the strips preincubated without Na^+ than in the control preparations. Likewise, the lactate/glucose ratio was higher in the strips preincubated in a Na^+-free medium than in those preincubated with Na^+.

Discussion

Preincubation of intestine strips in a Na^+-free medium (KRT/Man) affected substantially the respiratory activity of the tissue, when it was measured subsequently in a medium in which Na^+ was restored (KRT/Na). O_2 uptake was reduced but was not entirely suppressed. In the absence of glucose, O_2 uptake was inhibited by 19 % in the first hour; higher values of inhibition were attained when incubation was maintained for longer periods of time.

STAMPA (2, 20) had already observed that the reduction of the respiratory activity was proportional to the amount of Na^+ replaced in the incubation medium; he also showed that, if the tissue was maintained in a Na^+-free medium, a complete recovery of the O_2 uptake, after the Na^+ was restored to the medium, could not be achieved.

When glucose was absent, no significant differences were found in the lactate production at 1 hr and at 5 hr. These results suggest that the maximum capacity of the tissue to form lactate, under these conditions, was completed during the first hour; since no more glucidic stores seemed to be available, the differences could not be significant.

In the presence of glucose, O_2 uptake was also inhibited, although to a less extent than in the absence of sugar.

In the strips preincubated with Na^+-free medium, glucose utilization was inhibited by 41.1 % after 3 hr; after longer periods of time, however, no inhibition was observed. It has been shown (11) that, in the control experiments, most of the glucose present in the medium is utilized during the first three hours; therefore, glucose is

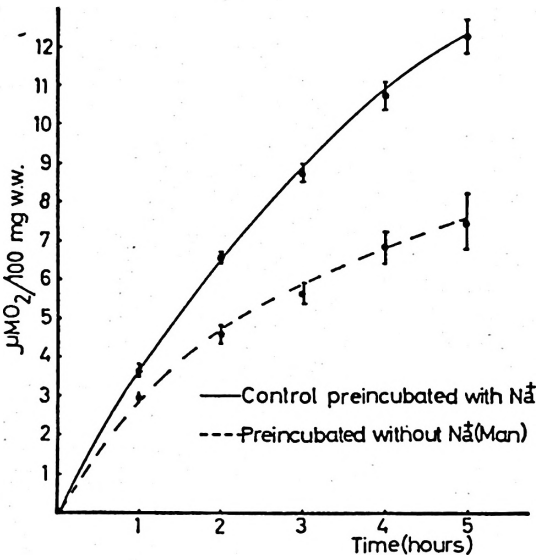

FIG. 1. Effect of preincubation without sodium (mannitol) on the O_2 uptake by strips of rat jejunum. The vertical bars represent the standard error of the mean. Data obtained from Table I.

no longer available to the tissue and can not be utilized during the subsequent hours. On the other hand, when the tissue is preincubated in a Na^+-free medium, glucose is used at a slower rate, so that the sugar is still available and utilized until the seventh hour. Thus, even when O_2 uptake is partially inhibited, the glucose present in the medium is still oxidatively metabolized; after the first three hours, the O_2 uptake of the preparations preincubated in Na^+-free medium can be stimulated by the substrate, whereas, in the control preparations, O_2 uptake can not be further stimulated because glucose is no longer available. This interpretation is supported by the results shown in Table I

TABLE II

Effects of the preincubation without sodium (mannitol) on the O_2 uptake, glucose utilization and lactate production by strips of rat jejenum

Preincubation of the strips in Krebs-Ringer-Tris/mannitol medium for 60 minutes. The control strips were suspended in Krebs-Ringer-Tris/Na^+ medium during the preincubation period. After preincubation, O_2 uptake, glucose utilization and lactate production were measured in Warburg flask in Krebs-Ringer-Tris/Na^+ medium, with 2,77 mM glucose. The mean values ($\bar{x}$) are expresed in mM/100 mg wet weight; ϵ = Standard error of the mean; N.° = number of test. Statistics according to Student's method

		Time (hours)					
		1	2	3	4	5	7
				O_2 uptake (μM/100 mg w.w.)			
Control	$\bar{x}$	4.25	8.26	10.56	12.01	14.64	17.34
	ϵ	0.10	0.15	0.33	0.62	0.70	1.07
	N.°	106	106	89	41	38	6
Preincubated without Na^+ (with mannitol)	$\bar{x}$	3.93	6.48	8.02	9.92	11.07	14.29
	ϵ	0.20	0.55	0.29	0.62	0.78	1.13
	N.°	33	26	32	18	11	9
Significance		$P < 0.2$	$P < 0.01$	$P < 0.001$	$P < 0.01$	$P < 0.001$	$P < 0.05$
Differences %		7.52	21.54	24.05	17.40	24.38	17.11
				Glucose utilized (μM/100 mg w.w.)			
Control	$\bar{x}$			5.86			6.38
	ϵ			0.19			0.45
	N.°			18			12
Preincubated without Na^+ (with mannitol)	$\bar{x}$			3.45			5.88
	ϵ			0.03			0.40
	N.°			18			12
Significance				$P < 0.001$			no sig.
Differences %				41.12			1.83
				Final Lactate (μm/100 mg w.w.)			
Control	$\bar{x}$			3.56			3.78
	ϵ			0.17			0.29
	N.°			32			12
Preincubated without Na^+ (with mannitol)	$\bar{x}$			3.39			5.60
	ϵ			0.20			0.48
	N.°			16			11
Significance				no sig.			$P < 0.001$
Differences %				4.77			36.50
				Lactate/glucose $\frac{\frac{1}{2}\ \mu M\ \text{Lactate Final}}{\mu M\ \text{Glucose utilized}}$			
Control				0.30			0.29
Preincubated without Na^+ (with mannitol)				0.49			0.47
Differences %				63.33			62.06

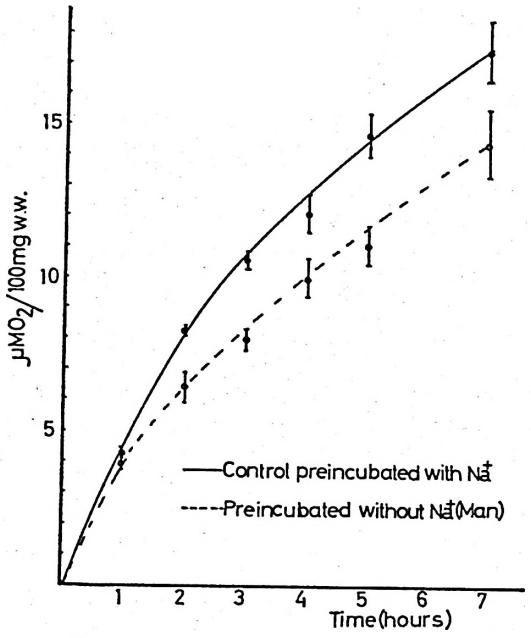

FIG. 2. Effect of preincubation without sodium (mannitol) on the O_2 uptake by strips of rat jejunum, measured with glucose in the medium. The vertical bars represent the standard error of the mean. Data obtained from Table II.

and II, where the effects of glucose on the O_2 uptake, in strips preincubated with and without Na^+, can be compared. When the strips were preincubated in a Na^+-free medium, the fraction of glucose that was not utilized in the first hours was able to stimulate O_2 uptake during the subsequent hours; on the other hand, in the control experiments preincubated with Na^+, O_2 consumption was scarcely stimulated, because substrate was no longer available after three hours.

In spite of the mentioned inhibition of glucose utilization, similar amount of lactate was produced during the first three hours, regardless of the amount of Na^+ present in the preincubation medium. Therefore, the lactate/glucose ratio was higher in the strips preincubated without Na^+. These results further support the evidence that the absence of Na^+ during one hour preincubation markedly disrupted the respiratory capacity of the tissue.

The metabolic disturbances induced on the tissue by the preincubation in a medium in which mannitol substituted for Na^+ may have important implications.

Mannitol has been used as a substitute for Na^+, at isosmotic concentrations, in experiments where the influence of Na^+ concentration on the active transport of several compounds was to be stablished (4). BOSACKOVÁ and CRANE (5) had observed in hamsters that the active transport of su-

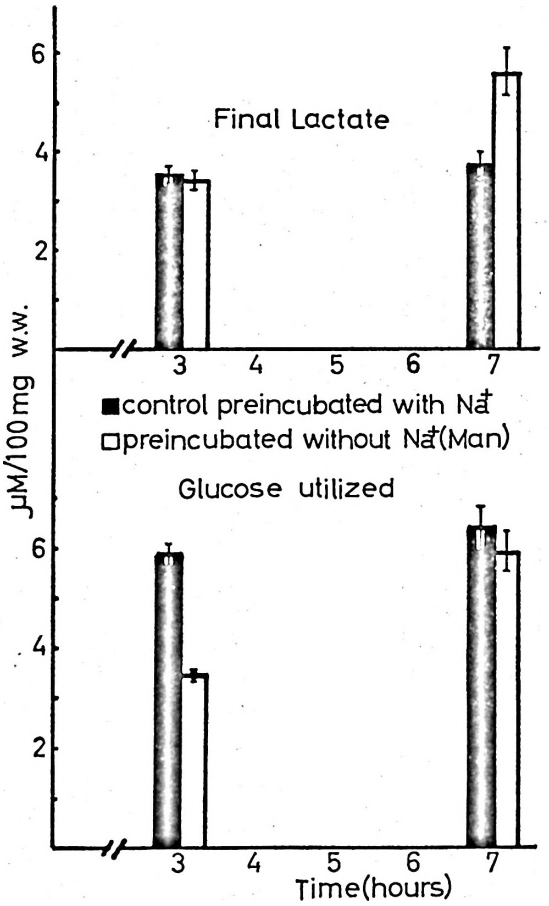

FIG. 3. Effect of preincubation without sodium (mannitol) on the glucose utilization and Lactate production by rat jejunum. The vertical bars represent the Standard error of the mean. Data obtained from Tabla II.

gars was reduced down to 50 % when the tissue was preincubated in a medium in which Na^+ was replaced by mannitol; this reduction, however, did not exist when other compounds were used. This rather peculiar action of mannitol could not be accounted for by its inability to penetrate inside the cell, because other non-penetrating compounds such as Tris and choline, which are ionic, did not show the same action of mannitol. Inhibition of the active transport of glucose has been also shown *in vivo* (17) when mannitol substituted for Na^+ in the solution to be absorbed. This inhibition was reversed when high concentrations of Na^+ were restored, not inmediately but after some recovery time. Similar effects were obtained, although to a less extent, if Na^+ was replaced by Li^+. These reports can, indeed, be correlated with the inhibition of O_2 uptake observed in the tissue incubated in KRT/Man (2, 20); and even more properly with the observations reported in this paper, which show that preincubation under those conditions modified the tissue so that, when it was subsequently incubated with KRT/Na^+, the O_2 uptake and glucose utilization were reduced, and the lactate/glucose ratio was increased. Actually, these alterations involve a reduction of the energy stores required by the tissue for active transport processes.

These results could alternatively be interpreted as the consequence of the inhibitory effect of the preincubation in a Na^+-free medium in the active transport of the substrate into the cell. This interpretation, however, can not be accepted because, first, partial inhibition of active transport would not be followed by modification of the lactate/glucose ratio, and second, O_2 uptake was also inhibited in the experiments performed on a glucose-free medium.

There are not sufficient data to explain adequately the metabolic alterations produced by the preincubation with KRT/Man. The absence of Na^+ may, indeed, be an essential factor, but, in order to stress the relevance of this factor, other compounds besides mannitol have to be tested. If the substitution of mannitol for Na^+, as it occurs in the active transport mechanisms, is the relevant factor that produces more important alterations, the phenomenon could be explained by the influence of the weak ionic strength of the KRT/Man medium. Modification of the ionic strength could bring about considerable changes to the ionic distribution between the intra and extracellular media. They, in turn, would modify either cellular structures, or the activity of enzymatic systems involved in the oxidative utilization of endogenous and/or exogenous substrates.

Summary

The influence of preincubation in a Na^+-free medium (mannitol substituting for Na) on the respiratory activity of intestine strips, measured during subsequent incubation in a Na^+restored medium, was evaluated. In the absence of glucose, O_2 uptake was inhibited. In the presence of glucose, O_2 uptake and glucose utilization were inhibited, and similar amount of lactate was produced. The lactate/glucose ratio in the strips preincubated in the Na^+-free medium was higher than in the strips preincubated with Na^+. These results further support the evidence that the absence of Na^+ during one hour preincubation disrupts the respiratory capacity of the tissue.

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