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# Oxygen Uptake by Jejunal Strips of Rat in the Absence of $Na^+$ (Replaced by Mannitol) [1]

by

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In a previous work (2) it was shown the effect of the lack of $Na^+$ in the medium (replaced by $Li^+$ or mannitol) on oxygen uptake by rat intestinal strips and mucosal fragments, over a period of one hour in the absence of external substrates. The oxygen consumption decreased with the degree of $Na^+$ substitution, attaining inhibition values of 32 % ($Li^+$) or 40 % (mannitol) with 100 % replacement of $Na^+$ for jejunal strips, and slightly higher values for mucosal fragments. These results were of interest since active transport of various substances by the intestine is dependent on the $Na^+$ concentration of the medium and on the energy derived to a great extent by aerobic metabolism. If the lack of $Na^+$ inhibits oxidative metabolism correlatively to the observed inhibition of $O_2$ consumption, consequently the availability of metabolic energy would diminish impairing active transport by an unspecific energy deficiency. This factor should be borne in mind when analizing the relationship between active transport and external concentration of $Na^+$.

The present work [3] attempts to make a more comprehensive analysis of charateristics of the inhibition of oxygen uptake due to $Na^+$ substitution. This involves the study of the reversibility of the process, what happens when sugars are present in the external medium, and the action of ouabaine.

## Material and Methods

The strips of rat jejune where prepared as is described in a previous paper (2). Between the death of the rat and the introduction of the strips in the Warburg flasks, a time of 5-10 minutes elapsed.

*Media:* KRT/Na: Krebs-Ringer-Tris. Composition and preparation as the «Krebs-Ringer-Phosphates» described in

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UMBREIT and col. (32), but using 0.2 M Tris-HCl buffer pH 7.4 (28) in replacement of the phosphate buffer.

KRT/Man: As KRT/Na, but with 300 mM Mannitol as a replacement for 154 mM NaCl, isotonic.

KRT/0.15 M Man: As KRT/Man, but with 0.15 M instead of 0.3 M Mannitol, hypotonic.

KRT/Man/77 mM Na: As KRT/Man, but with addition of 77 mM NaCl, hypertonic.

KRT/Man/154 mM Na: As KRT/Man, but adding 154 mM NaCl, hypertonic.

KRT/231 mM Na: As KRT/Na, but with 231 mM instead of 154 mM NaCl, hypertonic.

Sugars and ouabaine were dissolved in the media at the desired concentrations.

*Oxygen uptake determination:* It was made by the conventional manometric method of Warburg (32). The flasks contained 2.5 ml of the medium and the centre wells 0.2 ml of 10 % KOH. The apparatus was operated at 37° C, 100 oscillations/min. and amplitude of 3.5 cm, in an atmosphere of oxygen. The equilibration time was 10 minutes. Readings were taken at intervals of 15 or 30 minutes, during the experiment (1 a 8 hours duration). The oxygen uptake is expressed in microlitres per 100 mg of wet weight per 60 minutes. Normally in each determination 2 or 3 flasks were utilized under identical experimental conditions.

*Products:* Mannitol, D-Glucose and D-fructose were from Merck, Ouabaine from B.D.H. Tris from Schuchardt. The LiCl free from NaCl was supplied by Dr. P. Busquets from Probus. The absence of glucose in the mannitol was tested enzymatically with Clinton's reactive (18, 19).

## Results

1. AGEING OF THE PREPARATIONS. Jejunal strips of the same intestine were divided into three groups: one set was used for immediate determination, and the other two were utilized in determining the oxygen consumption, in the absence of external substrate, after maintining the strips during 1 or 2 hours in KRT/Na solution at 3-4° C. The conservation of the preparations in the cold prior to the determination progressively diminished

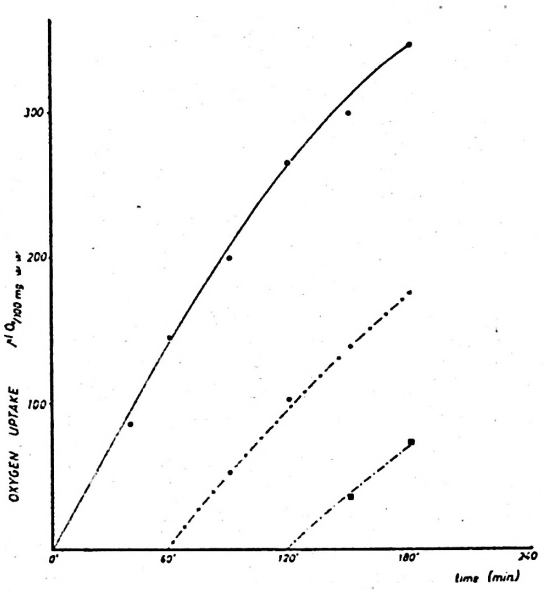


FIG. 1. $O_2$ uptake of rat jejune strips, immediately after its preparation (o), and after 60 min (•) or 120 min (□) of preincubation in KRT/$Na^+$ at 3-4° C

the respiratory capacity of the intestinal strips (fig. 1). To avoid errors by ageing, in all the sequent experiments the strips were used for determination of oxygen uptake just prepared.

2. INFLUENCE OF THE OSMOTIC PRESSURE. Besides the isoosmotic media KRT/Na and KRT/Man, other hypotonic (KRT/0.15 Man) or hypertonic media (KRT/Man/77 mM Na; KRT/Man/154 mM Na; KRT/231 mM Na) were also utilized.

The oxygen uptake of the preparations decreased in the order with the following

media: KRT/Na > KRT/231 mM Na > KRT/Man/77 mM Na > KRT/Man/154 mM Na > KRT/Man (fig. 2). In other words, the highest values were attained with the isotonic medium KRT/Na, with 154 mM $Na^+$ as oposed to the lowest values obtained with the isotonic KRT/Man, devoid of $Na^+$. The increase in osmotic pressure in the KRT/Na medium, which is due to an excess of NaCl or mannitol, diminished the oxygen consumption. The addition of $Na^+$ to the KRT/Man medium improves the tissue respiration. However, the increase obtained with 77 mM NaCl is greater than with a value of 154 meq of NaCl. In the last case the osmotic pressure is excessively high, and though the $Na^+$ concentration is the same as that in the normal KRT/Na medium, the $O_2$ consumption is almost so low as with the KRT/Man medium.

On the other hand it had been previously proved that the differences in $O_2$ consumption in a medium of KRT/Na, with 154 meq of $Na^+$, and in another KRT/Na with 77 meq of $Na^+$ (0.15 M of mannitol as substitute), were below 10 % (2). And the differences are insignificant when comparing tissue respiration in isotonic KRT/Man and in hypotonic KRT/0.15 M Man, during periods of 30 or 60 minutes, both the media being devoid of $Na^+$.

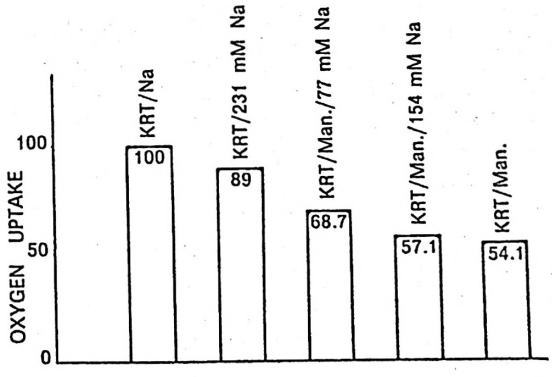


FIG. 2. $O_2$ uptake by jejunal strips in different media in 60 minutes, refered to that in KRT/$Na^+$ as 100

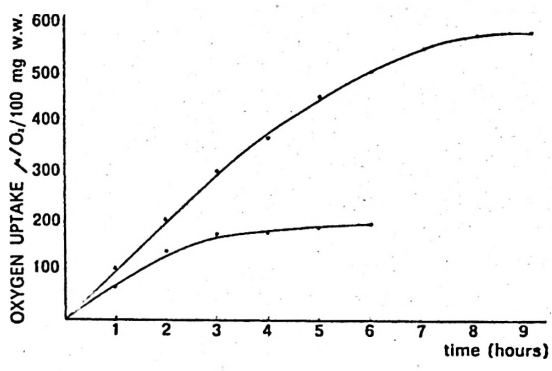


FIG. 3. $O_2$ uptake of jejunal strips in KRT/$Na^+$ (•) and in KRT/man (o)

3. EFFECT OF $Na^+$ ABSENCE IN EXPERIMENTS OF LARGE DURATION AND THE REVERSIBILITY DUE TO $Na^+$ REPOSITION. Preparations of jejunal strips from the same animal were incubated during periods of 8-9 hours in KRT/Na or KRT/Man media. In KRT/Na, the strips respired favourably over the experimental period, but displayed a gradual drop in $O_2$ consumption which appeared to cease towards the end of the period. Conversely, when the medium is KRT/Man the respiration was clearly lower and stops after 3 or 4 hours (fig. 3).

In another set of experiments, some jejunal strips were incubated over all the period in KRT/Na, or KRT/Man; other strips were put into KRT/0.15 M Man during the first 30 minutes, and then NaCl up to concentrations of 77 meq/l of $Na^+$ was added. The latter medium was devoid of $Na^+$ and hypotonic before the addition of $Na^+$ and isotonic after the addition. As figure 4 shows, the addition of $Na^+$ improves the $O_2$ uptake but the values remain well below those obtained with the KRT/Na medium.

4. OXYGEN CONSUMPTION IN THE PRESENCE OF EXTERNAL SUBSTRATE (GLUCOSE, FRUCTOSE) IN THE MEDIA WITH OR WITHOUT $Na^+$. D-glucose unlike D-fructose is transported actively by the epithelial

TABLE I

The influence of the absence of $Na^+$ on the increase of oxygen uptake provoked by addition of sugars to the medium

Strips of rat jejune were incubated during 60 min. in KRT/Na or KRT/Man media, with or without the presence of sugar (D-Glucose or D-Fructose). The increases in oxygen uptake produced by the addition of sugar are expressed, with the standard deviation, in per cent of the control values without sugar. In parenthesis, number of experiments

| Medium | Increase of oxygen uptake (%) by addition of | |
|---|---|---|
| | 2.77 mM Glucose | 5.55 mM Fructose |
| KRT/Na | 25.5 ± 2.6 (10) | 19.7 ± 2.6 (7) |
| KRT/Man | 16.0 ± 3.2 (8) | 12.7 ± 3.0 (8) |

cells (8). However both the sugars are well metabolized.

The presence of 2.77 mM glucose or 5.55 mM fructose in KRT/Na (Table I) causes a 25 or 19.7 % increase in $O_2$ consumption as compared with the control experiments without external substrate. The same substrates added to media without $Na^+$ (KRT/Man) increased $O_2$ consumption only to 16 or 12.7 % respectively (Experiments of 1 hour duration).

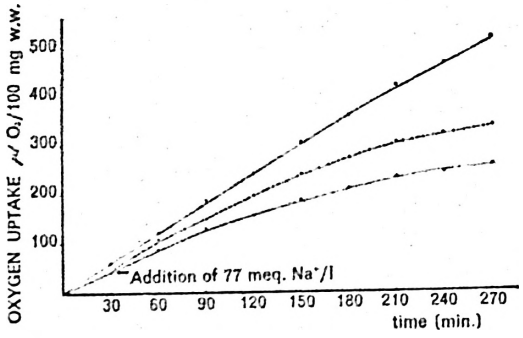


FIG. 4. $O_2$ uptake by jejunal strips in KRT/Na (●) and in KRT/Man (○). Effect of adding NaCl to reach a conc. of 77 meq $Na^+$/l after 30 min. incubation in KRT/Man (△)

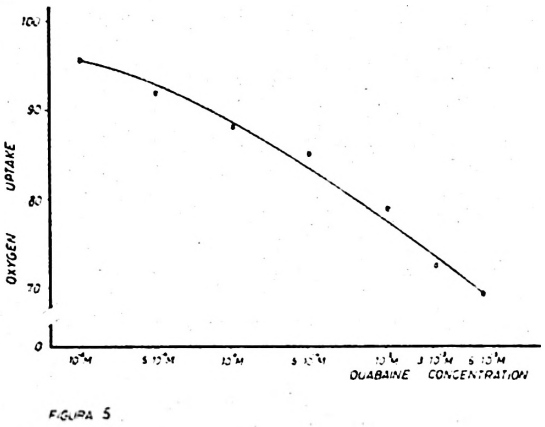


FIGURA 5

FIG. 5. Effect of ouabain on the $O_2$ uptake by jejunal strips in 60 minutes. Control = 100

Oxygen uptake with 2.77 mM glucose was 51.4 % less in KRT/Man than in KRT/Na. This represents a similar inhibition to that produced by the complete substitution of $Na^+$ in the absence of external substrate (2).

5. EFFECT OF OUABAIN ON OXYGEN CONSUMPTION. It is well known, that ouabain most probably affects the active transport of sugar (10) by inhibiting the active transport of $Na^+$ (16, 28). This concept has served as a basis to draw relationship between the active transport of $Na^+$ and the additional $O_2$ consumption required for this process.

It was, therefore, interesting to know the effect of ouabain on the oxygen uptake of jejunal strips in different conditions. Figure 5 illustrates the inhibition of $O_2$ consumption by ouabain ($10^{-5}$-$6 \times 10^{-3}$ M) when the strips are incubated in KRT/Na. To reach inhibition values of 20 %, a very high concentration of ouabain ($10^{-3}$ M) is needed.

If $10^{-3}$ M ouabain is present in a medium devoid of $Na^+$ (KRT/Man), no inhibition was observed.

Table II shows the action of ouabain on preparations incubated in KRT/Na, in the absence of external substrate, or in

TABLE II

The inhibitory effect of ouabain on the oxygen uptake of jejunal strips

The strips were incubated during 60 minutes in KRT/Na medium, with or without sugar, in the presence of ouabain. The per cent inhibition with its standard deviation is refered to the oxygen uptake in the same conditions but without ouabain. Number of experiments is shown in parenthesis

| Ouabain M | Inhibition (%) | | |
|---|---|---|---|
| | KRT/Na | KRT/Na + 2.77 mM Glucose | KRT/Na + 5.55 mM Fructose |
| $1 \times 10^{-5}$ | 4.0 ± 3.0 (5) | — | — |
| $5 \times 10^{-5}$ | 8.2 ± 1.7 (4) | — | — |
| $1 \times 10^{-4}$ | 11.4 ± 2.0 (7) | 10.4 ± 1.4 (5) | 9.3 ± 1.5 (4) |
| $5 \times 10^{-4}$ | 14.3 ± 1.7 (3) | 18.4 ± 1.7 (5) | — |
| $1 \times 10^{-3}$ | 20.8 ± 2.4 (10) | 24.2 ± 2.5 (10) | 20.4 ± 2.4 (9) |
| $3 \times 10^{-3}$ | 27.2 (2) | — | — |
| $6 \times 10^{-3}$ | 30.4 (2) | — | — |
| $1 \times 10^{-2}$ | — | 43.4 ± 3.3 (2) | 32.9 ± 1.1 (2) |

the presence of 2.77 mM glucose or 5.55 mM fructose. The inhibition of $O_2$ consumption is of the same order or somewhat higher when glucose is present in the external medium, than in the other conditions.

## Discussion

The normal $O_2$ uptake in KRT/Na medium, in our working conditions, was 89.2 $\mu$l $O_2$/100 mg w. weight/60 min. ($QO_2$ about 5.25), a value which is within the wide range found in the literature (1, 7, 12, 13, 15, 21, 25, 26, 33, 35, 36). As the respiratory capacity of the strips decreases with time (at 37° C or at 3-4° C), it was convenient to keep a constant time between the removal of the intestine and the beginning of the experiments to obtain comparative results.

Experiments of large duration (8-9 hs) have demonstrated that in a $Na^+$-free medium (Mannitol as substituting for $Na^+$, KRT/Man), $O_2$ uptake is remarkably low, as compared with that in a medium with $Na^+$ (KRT/Na). Moreover, the respiration ceases within 3-4 hours, much earlier than in the presence of $Na^+$. These results strongly suggest that total replacement of $Na^+$ by mannitol does not reduce $O_2$ uptake as a mere consequence of suppressing $Na^+$ active transport, but inducing an important alteration in the metabolic machinery of the tissues leading to the soon extinguishment of respiration.

Oxygen uptake is influenced by osmotic changes. When the isotonic medium KRT/Na is made hypertonic by adding mannitol or a $Na^+$ surplus, decreases the $O_2$ consumption. However, the addition of $Na^+$ to isotonic $Na^+$-free medium (KRT/Man) improved the $O_2$ uptake in spite of hypertony, but optimum values were reached at $Na^+$ levels that did not make the medium excesively hypertonic. On the other hand, there was not found a significative difference between tissue respiration in isotonic (KRT/Man) or hypotonic (KRT/0.15 M Man) $Na^+$-free media, at least during the first 60 minutes.

When after 30 minutes incubation in the $Na^+$-free KRT/0.15 M Man medium, NaCl is added up to 77 mM concentra-

tion, the respiration of the strips is ameliorated but it still remains very below the level obtained in a KRT/Na medium (fig. 4). This shows that the inhibition of $O_2$ uptake by incubation during 30 minutes in a mannitol medium without $Na^+$, is only in part reversibilized by adding $Na^+$, insinuating a durable alteration of the respiratory systems caused by that treatment.

In spite of some discrepant references (9, 13, 31), the presence of glucose or fructose in the medium stimulates oxygen uptake (1, 7, 25) as we have clearly confirmed. This stimulation is higher in mucosal (1) than in whole wall strips, on account of the higher metabolic activity of mucosa. The increase in $O_2$ uptake was greater with glucose than with fructose, even at twofold concentrations of the latter. This can be attributed to the more rapid penetration and accumulation of glucose within the cell, on account of the active transport.

Also when the strips are in mannitol medium (KRT/Man), without $Na^+$, the presence of sugars increases the $O_2$ consumption, however, to a lesser degree than in the medium with $Na^+$. In glucose this result is well understood because in the absence of $Na^+$, the active transport is abolished (3, 4, 5, 11, 14, 20, 22, 24) and much less substrate is available to be metabolized. But with fructose, a not actively transported and $Na^+$ independently transfered sugar (6, 14, 34), the result is striking. If the penetration rate of fructose into the cell is the same in KRT/Man as in KRT/Na and, on the other hand, the metabolic capacity of the tissue were not affected by the absence of $Na^+$ in the medium, the stimulation of oxygen uptake by adding fructose should be relatively higher when the strips are in KRT/Man than if they are in KRT/Na, since the corresponding values in the absence of exterior substrate are lower in the former than in the latter medium. However, what takes place is precisely the contrary. This is another argument leading to conclude that the lack of $Na^+$ (replaced by mannitol) by some way alters the respiratory systems of the cell.

Ouabain inhibits the oxygen uptake of jejunal strips incubated in KRT/Na. Nevertheless, it is a weak inhibitor, as $10^{-4}$ M or higher concentrations are required to observe clear effect, and at $6 \times 10^{-3}$ M, only a 30 % inhibition is attained. Similar orders of inhibition are obtained with glucose or fructose in the medium. In the absence of $Na^+$, $10^{-3}$ M ouabain has no effect, a result that may lead to consider that inhibition by the glucoside is not due to a direct effect on respiration, but by blocking the active transport of $Na^+$ (10, 27). However, perhaps this is not the case, because the concentrations of ouabain which block $Na^+$ active transport in different tissues and in the intestine (29, 30) are much lower than those inhibiting oxygen uptake by intestinal strips. Besides, the inhibitory effect is of the same order in the absence of external substrate than in the presence of glucose or fructose.

It is, on the other hand, interesting that the highest inhibition obtained with $6 \times 10^{-3}$ M Ouabain, a concentration at which one can expect a total block in the active transport of $Na^+$, remains well below the inhibition produced by the lack of $Na^+$ (mannitol as substituent), suggesting that this last condition ought impair somewhat more than $Na^+$ transport.

There appears therefore several results, which are not compatible with the interpretation that the decrease of oxygen uptake observed when $Na^+$ in the medium is replaced by mannitol may be attributed to the simple diminution in demand of energy just for the lack of activity of the $Na^+$ pump. On the contrary, they seem to require that this substitution, independent of the no functioning of the $Na^+$ pump, produces some alterations in the respiratory systems impairing the oxidative utilization of both endoge-

nous or exogenous substrates. These results are: *a*) in the absence of $Na^+$, respiration ceases much before that in media containing $Na^+$; *b*) after a period of incubation without $Na^+$, restitution of $Na^+$ enhances but does not reestablish normal oxygen uptake; *c*) suppression of $Na^+$ reduces the stimulation of oxygen consumption caused by the presence of fructose, a sugar whose entry into the cell should not depend on $Na^+$; *d*) high concentrations of ouabain which completely block active transport of $Na^+$ in different preparations, inhibit oxygen uptake in less proportion than the lack of $Na^+$ does.

The same conclusion of any injury in the respiratory systems produced by the lack of $Na^+$ (when replaced by mannitol) is also drawn by the observation of preincubated strips (1 hour) in KRT/Man, which later, on returning to a KRT/Na medium, exhibit a lesser degree of $O_2$ and glucose utilization (17).

When analizing the energetic relationship between transport of $Na^+$ and the required suprabasal $O_2$, with reference to differences in $O_2$ uptake according to the presence or absence of $Na^+$ in the medium, the posible alterations in the respiratory systems induced by the lack of $Na^+$ have to be considered, at least when the cation has been replaced by mannitol. The same consideration has also to be made when establishing quantitative relationship between the active transport of many substances (sugars, amino acids, etc.) by the intestine and the $Na^+$ concentration in the external medium, since a very low level or the total absence of $Na^+$ (replaced by mannitol), impairing the respiratory systems of tissues decreases the availability of energy necessary for the active transport.

## Summary

The $O_2$ uptake of jejunal strips has been determined in Krebs-Ringer-Tris media with or without the presence of $Na^+$. The total substitution of $Na^+$ produced a pronounced decrease in $O_2$ consumption, followed by respiratory cessation within 3 or 4 hours. This effect is only parcially reversed by the replacement of $Na^+$. The addition of glucose or fructose to a medium with $Na^+$, enhances consumption of $O_2$, but the increase is much lower when the sugars are added to a medium without $Na^+$ (replaced by mannitol). Ouabain, at very high concentrations ($10^{-4}$ M to $6 \times 10^{-3}$ M) inhibit the $O_2$ uptake in a medium with $Na^+$, independent of the presence or absence of glucose or fructose; no appreciable effect is found when the medium is $Na^+$-free. The highest inhibition reached with ouabain is much below those obtained by the suppression of $Na^+$ (mannitol).

The results lead to the conclusion that replacement of $Na^+$ by mannitol, does not only decrease $O_2$ uptake by the simple standstill of the $Na^+$ pump, but also inducing durable alterations in the oxidative metabolism of the tissues.

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