REVISTA ESPAÑOLA DE FISIOLOGIA, 29, 83-88. 1973

The Effect of Temperature and Absence of Sodium on the Oxygen Uptake, Water and d-Galactose Transfer by the Intestinal Sacs of Fish *Gobius paganellus L.**

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(Received on 31 January 1973)

L. HERRERA and R. JORDANA. *The Effect of Temperature and Absence of Sodium on the Oxygen Uptake, Water and D-Galactose Transfer by the Intestinal Sacs of Fish* Gobius paganellus *L.* Rev. esp. Fisiol., 29, 83-88, 1973.

Oxygen uptake and galactose and water transfer by everted sacs of intestine of the fish *Gobius paganellus* have been measured at 25, 30 and 37° C. The O_2 uptake increased with temperature, but it became stabilized between 30 and 37° C. The water transfer from mucosal to intestinal wall increased with temperature. A linear correlation between O_2 uptake and water transfer to tissue has been observed. The water transfer from mucosal to serosal decreased with temperature rises and became nil at 37° C. The active transport of galactose increased with the temperature reaching a maximum at 30° C, and started to diminish at 37° C. The absence of Na^+ in the incubation medium inhibited O_2 uptake, and water transfer to tissues and to serosal side. The galactose active transport was very strongly inhibited by the absence of Na^+.

Reports from many laboratories have shown that sugars and other solutes are actively transported by the fish intestine. It has been observed that in these animals there is a great variability regarding their behaviour with respect to active sugar transport and water transfer.

In some cases (13) it has been shown that there is no glucose transport. In others, the sugar transport is dependent on the oxidative metabolism (9, 10). However, this correlation has not been established in all cases (10). Active transport of substances by the fish intestine is a general function, very similar to that exhibited by

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

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other animals, but with peculiar characteristics related to their habitat.

In the references consulted there is no notice on the species used in the present work: *Gobius paganellus* L.* The interest of the results depends on the fact that it is a euryhaline and eurytherm fish. Its habitat is the intercotidal zone, where it remains isolated in small puddles during ebb-tide. In this time, due to solar radiation, the water of puddles increases its temperature up to 50° C and in some cases evaporates up to a third of its volume. This supposes a great concentration of salt. In these conditions the fish in question lives without apparent stress. It has bentonic customs and decreases its body temperature by contact with the bottom of the puddles.

The study of the transport mechanism is interesting in an animal that, being different from standard animals used in the laboratory, is subject to a high stress owing to its adaptation to a periodically changing environment.

This work studies the oxygen uptake, water and galactose transfer at different temperatures and the effect of the absence of sodium in the incubation medium on these parameters.

Materials and Methods

Male and female specimens of *Gobius paganellus* L. (8) have been employed, collected from *Punta del Hidalgo, Tenerife* (Spain). Until use, they were kept in a 300 l tank of sea water at 20 ± 2° C temperature. Specimens for experimental use usually measured 7-10 cm in length. After decapitation, the abdomen was opened, and the intestine removed and everted. The whole everted intestine was filled with incubation fluid (20).

The incubation medium was that of Krebs-Ringer (18) buffered with Tris-ClH. This medium was selected because it was the best suited for the tissues oxygen uptake. The oxygen uptake with this medium is within the values obtained for other marine species (12). In the cases in which a free sodium medium was used, the cation was substituted by Tris for osmotic effects (6).

The initial galactose concentration in the mucosal and serosal side was 1 mM. The final sugar concentration was measured by NELSON's method (11). The water transfer was calculated by weight difference. The O_2 uptake of the tissue was measured by the direct method of Warburg (19) in an oxygen atmosphere. In the incubation temperatures of 25, 30 and 37° C were employed. In all experiments the mucosal volume was 2.5 ml.

Results

Oxygen Uptake by the Intestine in vitro. Table I shows the results obtained for oxygen uptake in the everted intestinal sacs, at 25, 30 and 37° C, during 30 and 60 minutes, in Warburg flask incubation with a medium of KRT/Na^+-galactose 1 mM. The tissue shows a good respiratory level throughout the incubation time. The values obtained for O_2 uptake are similar to those of other marine teleostei (12).

Between 25 and 30° C the tissue increased its O_2 uptake in more than 100 % from 2.45 to 5.26 μM O_2/100 mg w.w./60 min. At 37° C its O_2 uptake, increased slightly though not significantly (5.81 μM O_2/100 mg w.w./60 min.).

Interestingly the O_2 uptake is similar to that obtained for rats in the same experimental conditions (1).

Above 30° C a stabilization of O_2 uptake was observed (Fig. 1).

The substitution of Na^+ by Tris in the incubation medium, at a temperature of 30° C, was observed to inhibit oxygen uptake 56 % with respect to the O_2 measured in the same experimental conditions but

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* Teleostei, Gobidae.

Table I. *Effect of sodium absence and temperature change on the O_2 uptake, galactose and water transfer by intestine sacs of* Gobius paganellus L.

The results are expresed in μM O_2/100 mg w.w. mM final concentration of galactose, net transfer of galactose in mg/100 mg w.w./60 minutes, water gain o fthe whole sacs and the intestinal wall is expressed in μl H_2O/100 mg w.w./60 minutes. Mean values with M.S.E. The medium was the Krebs-Ringer-Tris/Na^+ with 1 mM galactose. In the medium without Na^+ (KRT/Tris) this cation was substituted by TRIS. The differences % are the inhibitions for Na^+-absence in the medium. ( ) number of the animals used. Statistical signification by Student's t-method.

	KRT/Na^+			KRT/TRIS 30° C (12)	Diff. %
	25° C (18)	30° C (10)	37° C (12)		
O_2 uptake 30 minutes	1.13±0.09	2.22±0.15	2.94±0.17	1.10±0.06	—50.45 $P < 0.001$
O_2 uptake 60 minutes	2.45±0.19	5.26±0.18	5.81±0.25	2.30±0.10	—56.27 $P < 0.001$
Water gain of the sacs 60 minutes	83.37±9.40	72.73±8.88	70.87±5.48	21.88±2.47	—69.91 $P < 0.001$
Water gain of tissue 60 minutes	27.35±3.44	56.19±3.18	68.68±4.69	24.86±3.94	—55.77 $P < 0.001$
Galactose transport					
Mucosal final	0.97±0.04	0.90±0.01	1.17±0.06	1.15±0.07	
Serosal final	3.06±0.22	3.48±0.10	3.06±0.12	2.66±0.12	
Sf/Mf	3.08±0.18	3.84±0.06	2.62±2.34	3.34±0.10	—39.06 $P < 0.001$
Net transfer	0.0854±0.0117	0.1614±0.0118	0.0983±0.0141	0.0278±0.0040	—80.54 $P < 0.001$

with sodium in the medium. The result is similar to that found in rats (6), though in this fish it is very much accentuated.

Water transfer. The water gain of the sac and intestinal wall was measured by weight difference between the initial and final weight of empty and full sacs.

The weight increase of the sacs at different temperatures, after one hour of incubation, was found to be not significant (Table I). However there was a real variation in the gain of tissue weight: at 25° C the tissue increased its weight up to 27.35 μl/100 mg w.w.; at 30° C it increased up to 56.19 μl/100 mg w.w. This is a 100 % increase. From 30 to 37° C there was a little gain. This phenomenon is of the same order as that observed for oxygen uptake (Fig. 1).

The water transfer from mucosal to serosal (difference between water gain of sac and water gain of tissue) shifted with the temperature change, inversely from the O_2 uptake, and is almost nil at 37° C.

The absence of sodium in the incubation medium inhibited the tissue's water transfer some 55 % (it is of the same order as the inhibition of the oxygen uptake) and completely inhibited water transfer to serosal.

These results lead us to think that the water transfer somehow depends on oxidative metabolism.

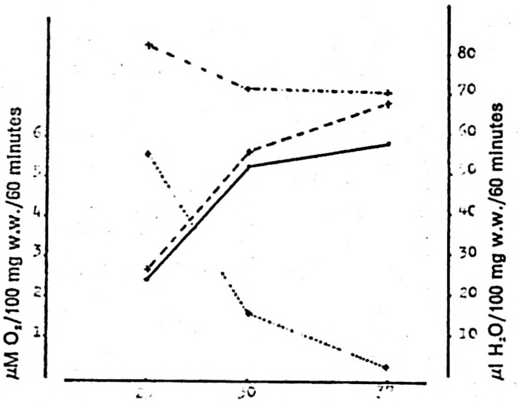

Fig. 1. *Effect of temperature on O_2 uptake and water transfer by everted sacs of* Gobius paganellus.
●: O_2 uptake. +: Water transfer. -.-.-.-: Water transfer from mucosal to whole sac. - - - -: Water transfer from mucosal to tissue. ....: Water transfer from mucosal to serosal side.

Active transport of d-galactose. Table I shows the results obtained for galactose transport in intestinal everted sacs.

After one hour of incubation at 25° C, the mucosal galactose concentration decreased, and the serosal increased. This indicates that there is active transport of galactose against a concentration gradient. The ratio serosal final concentration/mucosal final concentration (Sf/Mf) was 3.08 and the net transfer was 0.0854 mg/100 mg w.w./60 min.

This fact also occurred at 30° C, but the net transfer of galactose increased 80 %, and the Sf/Mf ratio rose to 3.84.

On the contrary, at 37° C the galactose active transport was inhibited. This fact is very different from that observed in O_2 uptake and water transfer, which did not change from 30 to 37° C. At this latter temperature an increase in the apparent mucosal galactose concentration was observed, which implies that some sugar should have come out of the tissue. This may explain the high molarity of the serosal medium. Intestinal tissues of other animals also liberate sugar to the medium (7).

The absence of sodium in the incubation medium (30° C) inhibited in 80 % the galactose transfer to serosal. Perhaps this inhibition is not total owing to a release of endogenous sugar from the tissue.

Discussion

Everted intestinal sacs of *Gobius paganellus*, when maintained in an ordinary saline medium with 1 mM galactose increased their O_2 uptake when the temperature increased from 25° C to 30° C, as is usual in all animal tissues.

Yet this phenomenon was not produced in our experiment, between 30 and 37° C. This striking fact supposes a saturation state.

As *Gobius paganellus* is a fish capable of resisting high temperatures in its environment, the fact that O_2 uptake does not follow temperature increase from 30° C upwards points to a protection mechanism against the exhaustion of cellular oxididizable substrates while the fish remains in a high temperature environment for long periods of time. To reach conclusive results however this phenomenon should be analyzed in different tissues, and more experiments be made in other poikilotherm and homeotherm animals.

When Na^+ is substituted by Tris, the O_2 uptake at 30° C is inhibited in 56 %. The effect of Na^+ absence on the O_2 uptake by rat jejunum has been studied in the laboratory (6). The inhibition of O_2 uptake cannot be explained only by the Na^+ pump supression, but there are other factors such as the biochemical respiratory mechanism which must be disturbed in a partially or non-reversible manner. This inhibition depends on the kind of substitute used to replace sodium. In rat jejunum the Tris is what less affects the O_2 uptake, while the *Gobius paganellus* intestine is greatly affected by this substitution. Indeed the Na^+ pump has to play a very important role in the intestine of this fish considering the high salinity that can be reached in the puddle waters.

The temperature dependence for water transfer has been shown in the rat intestine (15): at 18° C, there is no water transport; between 18 and 40° C the increase of transport is lineal with temperature and at 50° C it ceases. The O_2 uptake reveals the same relation with temperature. The water transfer is inhibited by phloridzin, iodoacetate and dinitrophenol. These facts manifest some dependence on oxidative metabolism. The water transfer generally shows dependence on temperature, O_2 uptake and sugar actively transported (16, 17).

Our results clearly express that there is a relationship between temperature, O_2 uptake and water transfer to tissue (no to serosal). This dependence supposes either an active water transport mechanism or an osmotic effect produced by active transport of other ions, sugars, etc.

In *Fundulus heteroclitus* (4) a relationship between increase of the serosal fluid and the presence of transportable sugars has been detected while it does not take place in non trasportable sugars. In *Gobius paganellus* we did not find that dependence with galactose transport. When the tissues water amount increased at 37° C, galactose transport to serosal and water transfer to serosal diminished. From 25 to 30° C galactose transport increased and water transfer to serosal decreased. All those phenomena are not connected in this fish.

In the absence of Na^+ the water transfer to tissue and serosal was inhibited. From the data presented it cannot be established if this is due to the inhibition of O_2 uptake or to the fact that the Na^+-pump might have been abolished.

Many authors have shown that there exists active transport of sugars by the fish intestine. *Fundulus heteroclitus* (5) transports d-galactose and this transport is temperature dependent up to a maximum of 37° C. The same fact is observed in *Tinca vulgaris* (3) and with relation to glucose transport in *Squalus acanthias* (2).

In our experiments the galactose transport increased with temperature, 100 % between 25° C to 30° C, coupled with a similar rise in O_2 uptake and water transfer to tissue. But while the O_2 uptake was constant between 30 to 37° C, the galactose transfer diminished. These results suggest that there is disconnection between energy production by metabolism and its utilization for active transfer of galactose.

At 30° C, the optimum temperature for maximum transport of galactose, an inhibition of 80 % in the transport of this sugar was observed using a Na^+-free medium. This is a clear evidence that this process is sodium dependent.

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