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TIME COURSE AND NATURE OF TEMPERATURE-INDUCED CHANGES IN SODIUM-GLUCOSE INTERACTIONS OF THE GOLDFISH INTESTINE

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SUMMARY

1. Glucose-evoked potentials measured at low incubation temperatures were found to be highly temperature dependent (phase 1), but less so at high incubation temperatures (phase 2) and acclimatization of an 8° C fish to 25° C resulted in the extension of phase 1 up to the environmental temperature of the fish. This change was only part of the mechanism controlling the acclimatization of sodium transport across the intestine.

2. The temperature at which the glucose-evoked potential changed from phase 1 to phase 2 was approximately equal to the temperature at which glucose began to raise the steady transmural potential of the intestine.

3. No changes in intestinal electrical parameters could be detected when fish, acclimatized to 8° C, were heated at 25° C for 15 hr, but after 20 hr at the higher temperature, acclimatization to the new temperature was complete.

4. Intestines from fish acclimatized to 8° C, but which had first spent 15 hr at 25° C and then 10 hr at 8° C, still behaved qualitatively like 8° C− intestines but the magnitude of the glucose-evoked potentials was slightly reduced.

5. It is suggested as a working hypothesis that acclimatization of the sodium-glucose interaction to different environmental temperatures involves the synthesis of new carrier molecules, qualitatively different from the old ones.

INTRODUCTION

When a goldfish intestine is incubated in physiologically normal saline, sodium is transported across the mucosa and appears later at the serosal surface (Smith, 1964*a*). This transport is associated with a transmural electrical potential which is stable (Smith, 1964*b*), but dependent on the previous acclimatization temperature of the fish (Smith, 1966*b*). Changes of this nature which follow those of environmental temperature appear to

regulate sodium transport but since the resting potential depends, in theory at least, on the rate at which sodium enters and leaves the mucosal cell, it is not profitable to speculate on the site or nature of this regulation solely on the basis of these measurements. Fortunately, however, the entry of sodium into the mucosal cell can be independently stimulated by glucose and the magnitude of this stimulation, which can always be induced but which is sometimes only transient, also depends on the temperature of the fish's environment, suggesting that the luminal membrane of the mucosa is one site where regulation takes place (Smith, 1966*a*, *b*).

It has been suggested that glucose exerts an allosteric control over part of the sodium entry into mucosal cells (Smith, 1966*b*). The object of the present work was to test this suggestion by a further study of changes in the sodium–glucose interaction caused by temperature acclimatization to see how these changes might contribute towards the regulation of sodium transport, and to examine the time period necessary to complete this aspect of acclimatization.

METHODS

Storage and temperature acclimatization of goldfish. Goldfish, obtained from Pet-Reks (Anglia) Ltd., Melbourn, Cambs., were kept initially at room temperature in aerated water for at least 1 week before use. They were then transferred as required to an acclimatization tank where the water was aerated, stirred and maintained at 8° C. Fish were left at this temperature for 2 weeks, then some time during the third week transferred to a second tank containing water at 25° C, where they were left for different periods of time. In some experiments goldfish acclimatized to 8° C and then transferred to 25° C were returned to water at 8° C before being killed. The fish were fed daily up to the time of the experiment and when maintained at 25° C were found to eat far more than at 8° C. In all cases food was given until the fish's appetite appeared to be satisfied. This was judged to be the case when extra food added to the tank was left uneaten for several minutes.

Preparation of intestinal sacs and the recording of transmural potentials. Goldfish measuring 10–15 cm (40–50 g), were decapitated and the intestine dissected free from adhering liver and connective tissue. The part of intestine used for the experiment was taken from the anterior part of the gut, about 5 cm below the intestinal bulb. The intestine was then everted on a stainless steel wire and tied to a polythene cannula. These manipulations took not more than 10 min and were carried out at room temperature in bicarbonate saline (Krebs & Henseleit, 1932) oxygenated with a mixture of 95 % O_2 + 5 % CO_2. Both the mucosal and serosal surfaces were washed with fresh bicarbonate saline, the intestine tied with thread to form a sac, and bicarbonate saline containing glucose (5 mg/ml.) placed inside the everted sac in contact with the serosa. The completed preparation was then transferred to 300 ml. of oxygenated bicarbonate saline at 20° C. Transmural potentials were recorded continuously with a Vibron electrometer through agar bridges containing 0·9 % NaCl. On some occasions a permanent record of changes in potential was achieved by taking the output voltage from the Vibron electrometer to a 'Xactrol' pen recorder (Ether Limited, Stevenage), suitably modified to give a full scale deflexion to 5 or 10 mV. All potentials were corrected for the junction potential measured at the beginning and end of each experiment. The resting potential of the sac increased gradually throughout the first 30 min incubation to reach a stable maximum after about 40 min. Only then was the sac transferred to the temperature gradient and the experiment proper begun.

Temperature gradient. The apparatus was a larger version of that described previously by Selwyn (1961). Its use with goldfish intestinal preparations has been described in detail elsewhere (Smith, 1966*b*). The only modification in the experiments reported below was to shorten from 10 to 8 min the period during which the intestine was kept in any one solution in the gradient since careful observation showed that the transmural potential became stable within this shorter time period.

RESULTS

Glucose-evoked potentials of intestines taken from fish acclimatized to 8 *or* 25° *C.* Intestines taken from fish kept previously in acclimatization tanks at 8 or 25° C for at least 2 weeks, were everted and glucose-evoked potentials measured at a number of different incubation temperatures. The results are shown in Fig. 1. The glucose-evoked potential was found to increase as the incubation temperature rose, at a rate which depended on the range of incubation temperature studied. At high incubation temperatures the glucose-evoked potential was less temperature dependent (phase 2) than at low incubation temperatures (phase 1). Acclimatization to 25° C increased the range of temperature over which the glucose-evoked potential showed phase 1 behaviour and the temperature of incubation where phase 1 changed to phase 2 was increased from 16 to 25° C. The glucose-evoked potential measured at an incubation temperature equal to the previous swimming temperature of the fish was only 0·4 mV for the 8° C intestine and 1·6 mV for the 25° C intestine. But the glucose-evoked potential measured at 25° C would have been still greater had no acclimatization occurred (2·3 mV for 8° C intestine) and the change from 2·3 to 1·6 mV was presumably an attempt to compensate for the higher environmental temperature of the fish. This compensation was only partly successful (type 3 regulation, Precht, 1958). The distinction between partial compensation of one mechanism concerned with intestinal sodium transport and perfect compensation of the complete system for sodium transport in the intestine will be discussed later. The partial compensation of the glucose-evoked potential was achieved by the relative displacement of the two curves (Fig. 1) without any significant change in the slopes of either phase 1 or phase 2.

Time course of temperature acclimatization of sodium transport across goldfish intestine. It has been established experimentally that, as the glucose-evoked potential changes from a phase 1 to phase 2 type behaviour, the steady potential in the presence of glucose exceeds that measured in its absence (Smith, 1966*a*). The degree of acclimatization can then be assessed by measuring the temperature at which glucose ceases to lower (or begins to raise) the steady transmural potential. This temperature was measured in intestines taken from fish which had been acclimatized to 8° C but then placed in water at 25° C for different periods of time before

being killed. Figure 2 shows clearly that the higher water temperature had no significant effect on this parameter of acclimatization when measured up to 15 hr after placing the fish in warm water. Moreover, acclimatization appeared to be complete by 20 hr; keeping fish for 16–18 days at 25° C caused no further rise in the temperature at which glucose ceased to lower the steady potential.

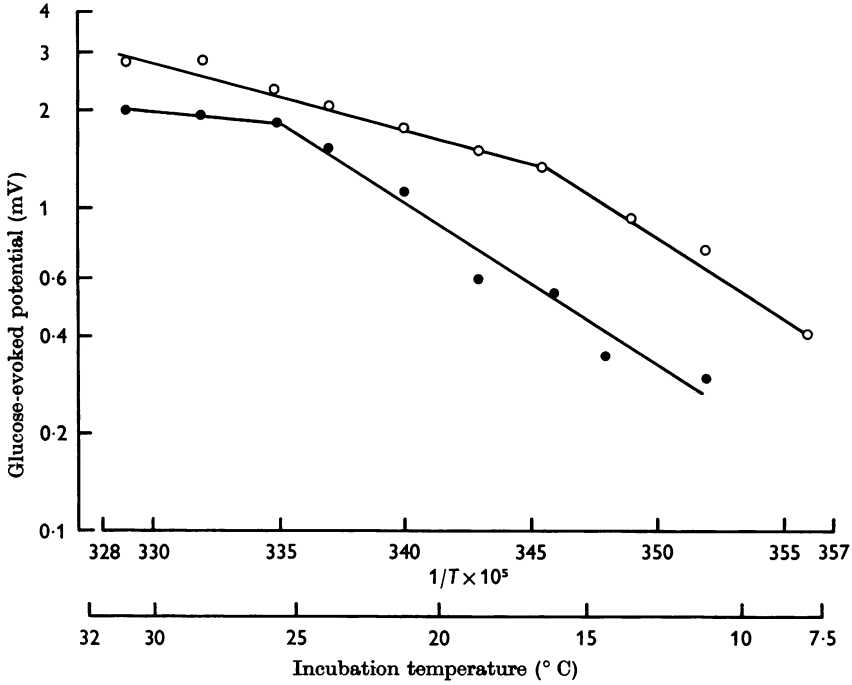

Fig. 1. Glucose-evoked potentials of goldfish intestine measured at different incubation temperatures. The environmental temperature of the goldfish had been maintained previously at 8° C, ○, and at 25° C, ●, for at least fourteen days before the experiment. Each point is the mean of 4–7 determinations. $1/T$, is the reciprocal of the incubation temperature measured in degrees absolute.

These changes appeared to coincide with changes in the glucose-evoked potential measured at different incubation temperatures. Results are shown in Fig. 3. There is no significant difference between 8° C intestines and intestines taken from fish acclimatized to 8° C after the fish had been swimming in water at 25° C for up to 15 hr. In a similar way results from 25° C intestines are the same as those from fish acclimatized previously to 8° C but kept in water at 25° C for 20–25 hr before beginning the experiment. Thus the detection of this aspect of acclimatization of sodium transport occurs only after a lag period of 15 hr and then acclimatization is completed within a remarkably short time. It would be extremely in-

teresting to know what, if anything, is happening during this initial lag phase but at the moment the techniques for observing changes appear too crude.

The short term effect of raising the environment temperature of goldfish on the glucose-evoked potential of the everted intestine. No change in the glucose-evoked potential of goldfish intestine can be detected 15 hr after raising the water temperature of an 8° C acclimatized fish to 25° C, yet 5 hr later

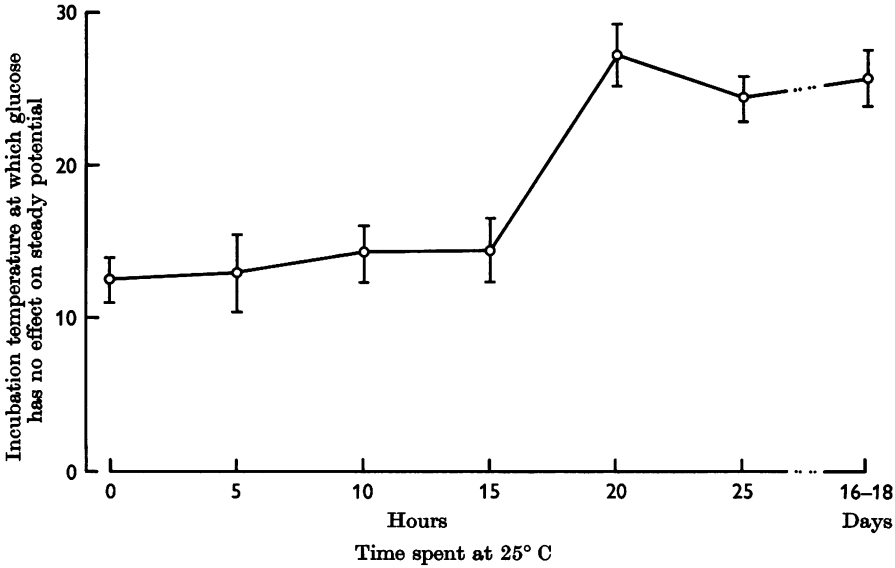

Fig. 2. The temperature of incubation where glucose no longer changes the steady transmural potential of goldfish intestine measured during the period of acclimatization of goldfish from 8° C to 25° C. At incubation temperatures below the line, glucose lowered the steady potential while at higher incubation temperatures the effect of glucose was to raise the potential. Each point gives the mean of four experiments ± S.E.

the process of acclimatization is complete. Which is critical—the time period 15–20 hr after raising the temperature of the fish, or the actual temperature of the water during this 5 hr period? If changes were induced early in the period of acclimatization they might continue despite removal of the temperature stimulus, in which case the fish intestine would be expected to exhibit a 'memory' of its previous experience and behave as if acclimatized to 25° C, although swimming at some new temperature. To see if this could occur, fish acclimatized to 8° C were kept at 25° C for 15 hr and then returned to water at 8° C for a further 10 hr. Glucose-evoked potentials were then determined with these intestines at a number of different incubation temperatures. Figure 4*b* shows the steady potentials, with and without glucose present, measured at the different incubation

temperatures. The intestine still behaved as an 8° C intestine with glucose beginning to increase the steady potential at temperatures greater than 16·1° C. The mean values of three experiments, where the glucose-evoked potentials were measured, are shown in Fig. 4*a*. Phases 1 and 2 of the glucose-evoked potential are as for an 8° C intestine although the absolute values are slightly lower.

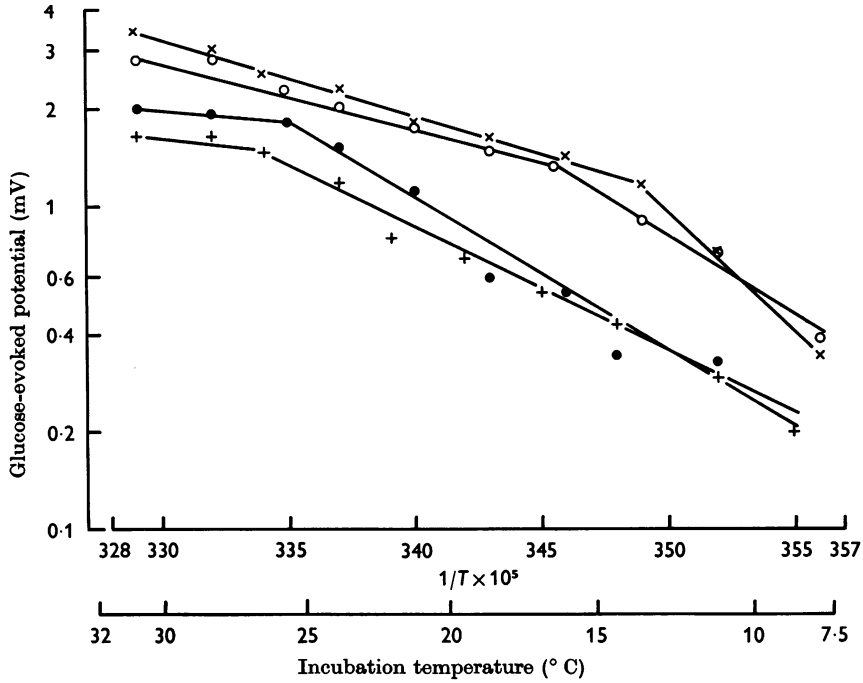

Fig. 3. Glucose-evoked potentials of goldfish intestine measured at different incubation temperatures. The intestines were taken from fish fully acclimatized to 8° C, ○, (seven experiments); acclimatized to 8° C but then kept at 25° C for 5–15 hr, ×, (twelve experiments); acclimatized to 8° C but then kept at 25° C for 20–25 hr, +, (eight experiments) and acclimatized previously to 8° C but then kept at 25° C for 16–18 days, ●, (four experiments). $1/T$, as in Fig. 1.

DISCUSSION

Previously it was shown that the absolute glucose-evoked potential of goldfish intestine depended on the acclimatization temperature of the fish (Smith, 1966*b*). Raising the acclimatization temperature lowered the glucose-evoked potential measured for any incubation temperature within the range 7–30° C. Since the glucose-evoked potential is an immediate event, it has been taken to represent the initial rate at which a carrier situated in the luminal membrane can move sodium into the mucosal cell. Any change in this carrier affecting sodium entry will be immediately

apparent as a change in the glucose-evoked potential. The present study was concerned with only two acclimatization temperatures, 8° C and 25° C, values well within the range of temperature tolerated by the fish (Fry, Brett & Clawson, 1942), yet sufficiently different to cause marked changes in the observed effect. Discrepancies in individual measurements were minimized by using mean results from a large number of fish.

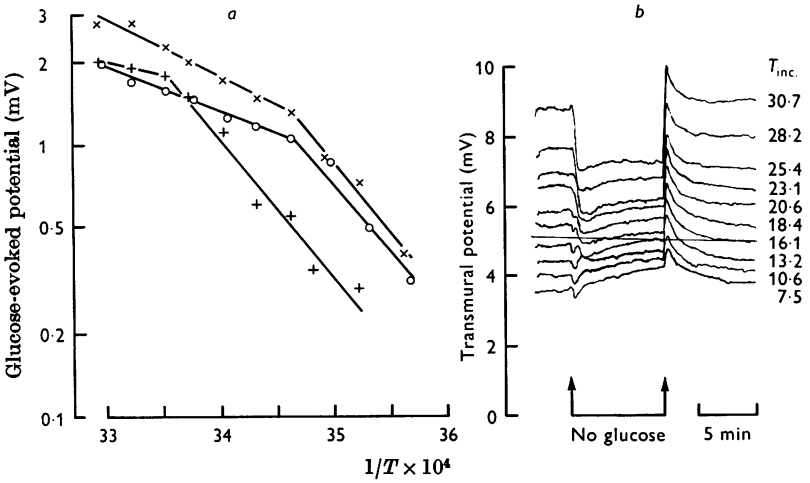

Fig. 4. Potential recordings of goldfish intestines taken from 8° C acclimatized fish after swimming at 25° C for 15 hr and then at 8° C for a further 10 hr. (*a*) Compares the glucose-evoked potentials of these intestines, ○, (three experiments) with those from fish fully acclimatized to 8° C, ×, or 25° C, +, measured at different incubation temperatures. $1/T$, as in Fig. 1. (*b*) Shows the record of transmural potential throughout an experiment using the intestine taken from a partly acclimatized fish. The horizontal line marks the temperature (16·1° C) above which glucose raises the steady transmural potential. $T_{inc.}$ is the incubation temperature.

Changes in the glucose-evoked potential, caused by acclimatization to a high temperature, are in a direction such as to stabilize sodium transport at its previous level but they only compensate partially for the change in environmental temperature (type 3 regulation; Precht, 1958). This type of regulation is common in poikilotherms (Prosser, 1964) but the interesting point here is that the steady potential, measured in the presence of glucose, regulates perfectly over the same temperature range (Smith, 1966*b*); type 2 regulation (Precht, 1958), so that more than one mechanism must change to regulate sodium transport across the mucosal cell. It follows that conclusions about the partial or complete acclimatization of an organism to a new temperature, if based on measurements of only one parameter, must be tentative only.

Speculations about the way in which the sodium carrier in the mucosal

membrane changes to regulate the influx of sodium have been connected with the conception that this carrier has allosteric properties (Smith, 1966*b*). Thus the carrier is assumed to be permanently switched on by glucose at high incubation temperatures but only partially switched on at low temperatures, the degree of partial activation depending on the incubation temperature chosen. Acclimatization to a high temperature results in a carrier which has more resistance to the effector action of glucose. This change could probably be achieved by the synthesis of new carrier molecules qualitatively different from the old ones. A simple numerical reduction of carrier molecules at the high acclimatization temperature would not account for the observed results. Some evidence for this view is that puromycin stops the acclimatization of glucose-evoked potentials normally induced by a change in the environmental temperature (unpublished observations). This emphasizes that it is synthesis, not degradation, of carrier molecules which is necessary to complete this aspect of acclimatization.

When fish acclimatized to 8° C swim at 25° C for 15 hr, their intestines still behave like those from a fish fully acclimatized to 8° C, yet 5 hr later the acclimatization to the higher temperature is complete. Rapid changes have also been noted when oxygen consumption is measured in goldfish during acclimatization to different temperatures (Baudin, 1932; Freeman, 1950; Klicka, 1965). In these cases an overshoot precedes the new stable level of oxygen consumption. There may be a connexion between changes in oxygen consumption of the goldfish and the changes in glucose-evoked potentials recorded here, but this was not investigated.

It was hoped, perhaps naïvely, that replacing a fish acclimatized to 8° C in water at 8° C after a 15 hr interval in water at 25° C might trick it into producing its 25° C type carrier while swimming at 8° C. This did not happen and the fish intestine behaved qualitatively as if acclimatized to 8° C. However, there was some suspicion that this procedure had a quantitative effect, reducing the absolute glucose-evoked potential at all temperatures of incubation. The initial lag period of 15 hr followed by the extremely rapid acclimatization of the glucose-evoked potential within 5 hr, can best be explained in terms of enzyme synthesis if this initial period were to represent the time necessary for induction of a new carrier. But the synthesis of new carrier molecules starting immediately after a rise in the body temperature of the fish, the rate of synthesis increasing as an exponential function, is an adequate alternative explanation which cannot be dismissed on the present findings.

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