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INFLUENCE OF TEMPERATURE ACCLIMATIZATION ON SODIUM–GLUCOSE INTERACTIONS IN THE GOLDFISH INTESTINE

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SUMMARY

1. Transmural potentials across goldfish intestines *in vitro* were found to depend on the acclimatization temperature of the fish. At any incubation temperature potentials were lower in fish kept previously at a high temperature, and if the transmural potentials were recorded at incubation temperatures equal to the previous acclimatization temperatures the values remained constant from 8 to 30° C. The glucose-evoked potential was also reduced by previous acclimatization of the fish to a high temperature.

2. As the sodium concentration was reduced the steady transmural potential increased and later fell in proportion to the low external sodium concentration, but the glucose-evoked potential fell as soon as the sodium concentration was reduced below 140 mM. Similar changes were seen with intestines taken from fish acclimatized to a high temperature but both the steady-state potential and the transitory glucose-evoked potential were more dependent on the external sodium concentration.

3. The maximum glucose-evoked potential depended on the concentration of glucose used and temperature acclimatization had no significant effect on this relation. The steady potential was lower in the presence of glucose at low incubation temperatures but higher at higher incubation temperatures, and the temperature at which glucose ceased to inhibit depended on the previous acclimatization temperature. Glucose also lowered the steady potential, whatever the previous acclimatization temperature, when the external sodium concentration was low.

4. The inhibitory effect of glucose on the steady potential of an intestine taken from a 30°-acclimatized fish could be abolished by lowering the external concentration of glucose from 27 to 16 mM.

5. Intestines taken from fish acclimatized to 3° C gave variable results.

6. It is concluded that sodium moves across the luminal membrane of the goldfish mucosa attached to a carrier which can exist in one of two forms. It is changes in this postulated carrier which serve to stabilize

sodium transport at different acclimatization temperatures. Changes in the concentration of this postulated carrier may also occur and function in the regulation of sodium transport, particularly at acclimatization temperatures below 15° C, where the switching of the carrier does not operate.

INTRODUCTION

It has been known for a long time that some fish, within a period of several days, can change their physiology to match the temperature of their environment. It is also well established that when fish are kept in water at various temperatures they choose to swim at a particular temperature (Fisher & Elson, 1950). Temperature acclimatization is therefore only likely to be of importance where behavioural changes are absent or no longer effective in regulating the over-all metabolism of the fish. However, when acclimatization does take place, it often changes subsequent behavioural responses and fish then choose to live at higher or lower temperatures depending on the nature of their previous acclimatization (Fry, 1947).

There is little doubt that a behavioural change occurs as the immediate response to sensory stimulation of the central nervous system. What is less clear is the cause and nature of changes which occur over the longer time period associated with temperature acclimatization. The long-term effect of changing temperature may well be to change the concentration of enzymes concerned directly or indirectly with the maintenance of homeostasis. It has been suggested that such changes could be due to the induction of enzyme synthesis through the action of hormones or by the provision of substrates for the enzyme in question (Knox, 1958). It is also possible to imagine an alteration in the structure of enzymic protein so as to change its energy of activation which would then affect subsequent biochemical reactions (Prosser, 1958). It was decided to investigate sodium–glucose interactions in the intestine of goldfish to see if any changes took place as a result of temperature acclimatization and to try to distinguish between changes in concentration and changes in the structure of the enzyme(s) responsible for this interaction.

Fish show a seasonal variation in their response to temperature and goldfish have been reported to change their susceptibility to both high and low temperatures with the time of year (Hoar, 1955). Trout also change their behavioural response to different temperatures in the autumn and spring, immediately before obvious changes in the temperature of the environment (Sullivan & Fisher, 1953). For this reason experiments reported in this paper were confined to a period of 14 weeks between the end of November and the beginning of March.

METHODS

Storage and temperature acclimatization of goldfish. Goldfish which were delivered from outside ponds were kept at room temperature in aerated water for at least 1 week before use. The water temperature in this holding aquarium varied from 10 to 15° C during the 14 weeks of experimentation. Fish were transferred periodically from this large aquarium to several acclimatization tanks where the water was aerated, stirred and maintained at a number of different constant temperatures. In some cases these temperatures were the final temperatures of acclimatization (12–20° C), while in others (3–8, 25–30° C) acclimatization was achieved by a two-stage process; fish were held initially at 12 or 20° C for 1 week and then kept at their final temperature of acclimatization for a further 2 weeks. All fish were killed after being kept at their final acclimatization temperature for at least 2 and not more than 3 weeks. The volume of water per fish during the period of acclimatization was 16 l.

Food was added to the tanks until the fish lost interest in feeding. In practice, goldfish maintained at the higher temperatures ate far more than those kept in the cold.

Preparation of sacs and recording of transmural potentials. The methods for everting goldfish intestine and recording transmural potentials were as described previously (Smith, 1966). The manipulations necessary to evert the intestine were carried out in oxygenated bicarbonate saline (Krebs & Henseleit, 1932) at room temperature. Pieces of intestine were taken from the anterior intestine, about one third the total distance from the intestinal bulb to the rectum. This was the area shown to support a high potential and maximal transport of glucose (Smith, 1965). Electrical potentials were recorded initially from sacs held in bicarbonate saline at 20° C. Thirty to 40 min later the potential was judged to be maximal and unchanging and the experiment proper was begun.

Temperature gradient. The apparatus used to maintain a series of different constant temperatures was as described by Selwyn (1961). It consisted of an ice-bath attached to a heated bath by a number of copper rods. The temperature of the heated bath was thermostatically controlled to maintain a 7–31° C temperature gradient along the copper. The rods had holes drilled in them at intervals. Tubes full of oxygenated saline rested in these holes and equilibrated with the temperature of the rod at that point of the gradient. There were two holes at any one temperature and ten different temperatures within the gradient. The cannulated everted sac was placed in a Perspex holder which fitted over the top of these tubes. This holder also held the agar bridges and a fine oxygenator and all could be lifted clear of one tube and placed in another within 5 sec.

RESULTS

Effect of acclimatization to high temperature on the transmural potentials of the goldfish intestine. Everted sacs of goldfish anterior intestine, taken from fish acclimatized to 12° C (12° intestine) or 30° C (30° intestine), were placed in a temperature gradient and the transmural potentials recorded in the presence or absence of glucose. The results are shown in Fig. 1. There are some similarities between the intestines treated in these two ways. The steady state potential with or without glucose increases as the temperature of incubation is increased and the rate of increase is more pronounced when glucose is present. Also both the preparations show increases in potential when transferred from glucose-free to glucose-containing media. But here the resemblance ends and in fact the differences between the two intestines are much more noticeable. The changes

in potential on moving to a glucose-free medium for the 30° intestine are the complete opposite from those for the 12° intestine, and though both show a glucose-evoked potential, that of the 12° intestine is well maintained but that of the 30° intestine is seen for only a few seconds. Finally, in any instance, the transmural potential of the 30° intestine is less than that of the 12° intestine. Further examination of Fig. 1 shows that at low incubation temperatures the 12° intestine maintains a higher steady-state potential in the absence of glucose than with glucose present, but at higher

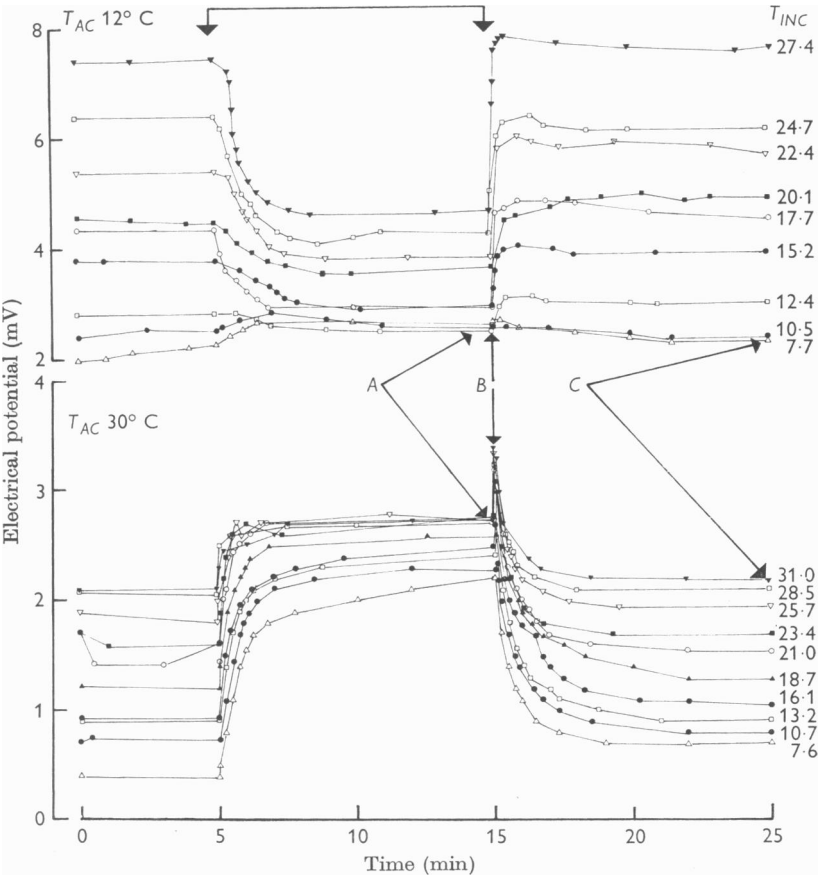

Fig. 1. Effect of glucose and temperature acclimatization on the transmural potentials of goldfish intestines measured at different incubation temperatures. T_{AC}, acclimatization temperature; T_{INC}, incubation temperature. Everted sacs were placed in glucose-free bicarbonate saline medium during the time period enclosed by the arrows. *A*, steady-state potential with no glucose; *B*, glucose-evoked potential, fleetingly seen in the intestine from the fish acclimatized to 30° C; *C*, steady-state potential with glucose present. The scale of transmural potential is different for the two acclimatization temperatures.

incubation temperatures the reverse is true. To understand the meaning of these several changes, three parameters were studied in detail in fish adapted to a series of temperatures between 3 and 30° C. These parameters, shown in Fig. 1, were: (*A*) the steady-state potential with no glucose in direct contact with the mucosa; (*B*) the glucose-evoked potential, and (*C*) the steady-state potential in the presence of glucose.

Effect of changing incubation temperatures on the glucose-evoked potentials of intestines taken from goldfish acclimatized to different temperatures. The change in potential on moving an everted sac from a glucose-free to a glucose-containing medium was measured at ten different incubation temperatures. The results obtained from goldfish acclimatized to three different temperatures are shown in Fig. 2. In all cases the glucose-evoked potential rose sharply as the temperature of incubation was

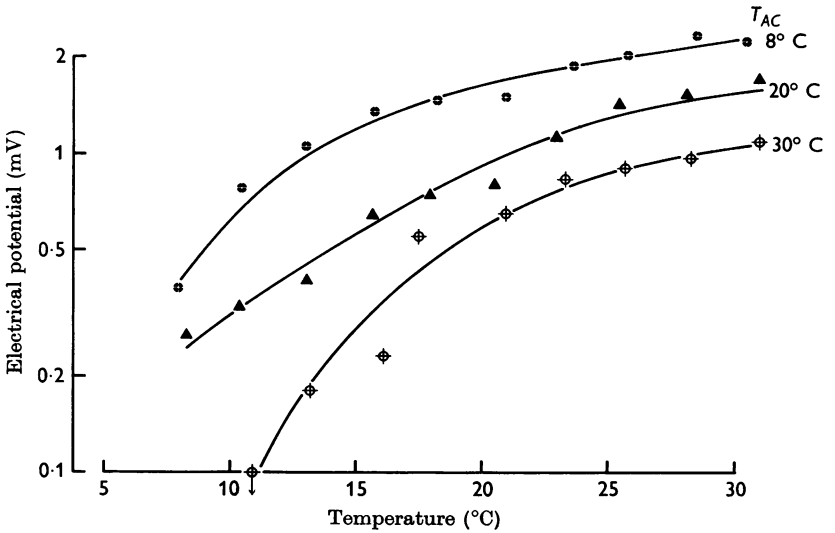

Fig. 2. Effect of temperature acclimatization on the glucose-evoked potentials of goldfish intestinal sacs recorded at different incubation temperatures. T_{AC}, acclimatization temperature: ⊕, 30°; ▲, 20°; ⇕, 8° C. ↓, less than value. Each point is the mean of three determinations.

raised, but at higher temperatures of incubation this rise was less pronounced. The results have been plotted as a series of convex curves but could be plotted as two straight lines of different slopes (Smith, 1965). Acclimatization to higher temperatures caused a translation of the curves to the right and downwards. There was no evidence for a change in Q_{10} values between the different temperatures of acclimatization. Similar experiments on other goldfish acclimatized to temperatures within the range 8–30° C showed intermediate translations of their curves but with

fish acclimatized to 3° C the curve resembled that of the 30° intestine in height and shape, the only difference being a translation to the left so that in this respect it resembled the 8° intestine. As later experiments will show, other goldfish acclimatized to 3° C sometimes did and sometimes did not show a large glucose-evoked potential when measured at 25° C. These anomalies may be due to the fish approaching some limit to adaptation. This variation was not found at the other temperatures of acclimatization.

Effect of temperature acclimatization on the steady-state potentials measured at different incubation temperatures. The steady-state potential in the presence of glucose, measured for any one everted sac of goldfish anterior intestine, was dependent on the temperature of incubation. The curve relating transmural potential to the temperature of incubation consisted of a rapidly rising phase and a slowly rising phase. The effect of temperature acclimatization on the relative importance of each phase is shown in Fig. 3. Here the slowly rising phase is drawn as a dotted line, the sharply rising phase as a solid line and the heavy interrupted line running across the figure is the transmural potential which is recorded when a goldfish intestine is incubated at the same temperature as its temperature of acclimatization. Temperature acclimatization causes a shift in the temperature at which the rapidly rising phase of the transmural potential changes to the more slowly rising phase. This shift is limited to temperatures of acclimatization above 15° C and in these cases the movement is in the same direction as the temperature of acclimatization. Below an incubation temperature of 15° C the rate of change of transmural potential with incubation temperature is always in the sharply rising phase. The process of temperature acclimatization produces a constant transmural potential when measured *in vitro* at the temperature of acclimatization (range 8–30° C). The transmural potential is changed by acclimatization to low temperatures (8–16° C) and the mechanism for this regulation would appear different from that seen at higher acclimatization temperatures. The goldfish adapted to 3° C must be considered separately. Results show a similar pattern to other fish adapted to temperatures of 16° C and below but there is evidence of a general fall in the rate of sodium transport.

Figure 3 can be condensed to represent fish acclimatized to temperatures from 25 to 30 and from 3 to 16° C. These mean curves can then be compared with similar ones determined with no glucose present. These results are shown in Fig. 4. Both the steady-state potentials with and without glucose inflect at about 15° when determined for the 3–16° intestines but this is not seen for the 25–30° intestines. The slopes of the steady-state potentials without glucose are very similar except for the sharply rising phase in the 3–16° intestines over the incubation tem-

perature range 7–15° C. Also the slopes of the steady-state potentials with glucose are similar, except for the slowly rising phase in the 3–16° intestines over the incubation temperature range 15–30° C.

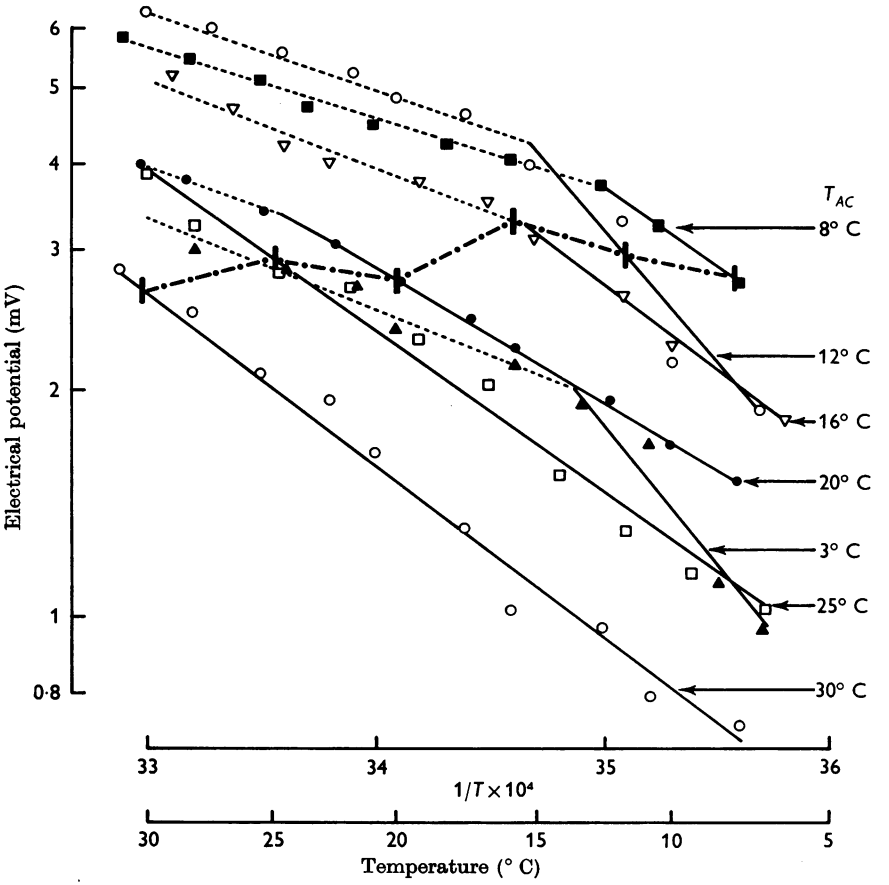

Fig. 3. Effect of temperature acclimatization on the steady-state transmural potentials of everted preparations of goldfish intestine measured at different temperatures of incubation with glucose present. T_{AC}, as in Fig. 1; $1/T$, reciprocal of the incubation temperature measured in Å. The slopes of the curves are represented as two lines, the continuous line shows the first slope obtained at lower incubation temperatures and the dotted line shows the second slope obtained at higher incubation temperatures. The heavy interrupted line gives the transmural potentials of everted sacs when incubated at their acclimatization temperatures. Each point is the mean of three determinations.

Effect of sodium concentration on the glucose-evoked potential of goldfish intestines taken from fish acclimatized to different temperatures. Goldfish were acclimatized to three temperatures (3, 13 and 30° C) and the change in potential, seen when intestinal sacs were moved from glucose-free to

glucose-containing media, was recorded for a series of sodium concentrations. Potassium chloride was substituted for sodium chloride. No changes were made in the ionic composition of the medium bathing the serosal surface of these sacs. The incubation temperature was kept constant at 25° C throughout the experiments. Results are shown in Fig. 5.

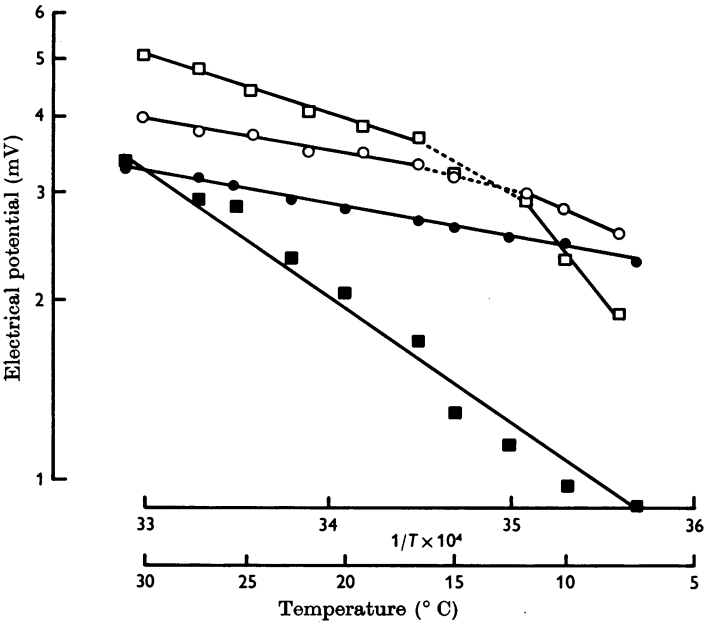

Fig. 4. Steady-state transmural potentials determined with and without glucose at different incubation temperatures for two ranges of acclimatization temperature. Open symbols are for temperatures of acclimatization, 3–16° C, and closed symbols for acclimatization temperatures, 25–30° C. ○—○ and ●—●, no glucose; □—□ and ■—■, glucose present. Each point is the mean of 6–9 values. $1/T$, as in Fig. 3.

The curves obtained for fish acclimatized to 3 and 13° C are very similar in shape. There was a slow fall in the glucose-evoked potential as the sodium concentration was reduced to 50 mM and then a rapid fall over the sodium concentration range (50–15 mM). The apparent affinity of sodium for the sodium–glucose interaction was greater in fish acclimatized to 3° than in fish acclimatized to 13° C, but the significance of this is doubtful in view of the variability of the results. Fish acclimatized to 30° C gave a small glucose-evoked potential even when measured at high sodium concentrations and as the sodium concentration was reduced below 140 mM the potential fell rapidly so as to be barely detectable at a sodium concentration of 50 mM. The apparent affinity of sodium for the sodium–glucose interaction was much reduced by acclimatization to 30° C. The

differences between 30° and 3–13° intestines are thought to be significant and of some importance in understanding the mechanism of temperature acclimatization in this tissue.

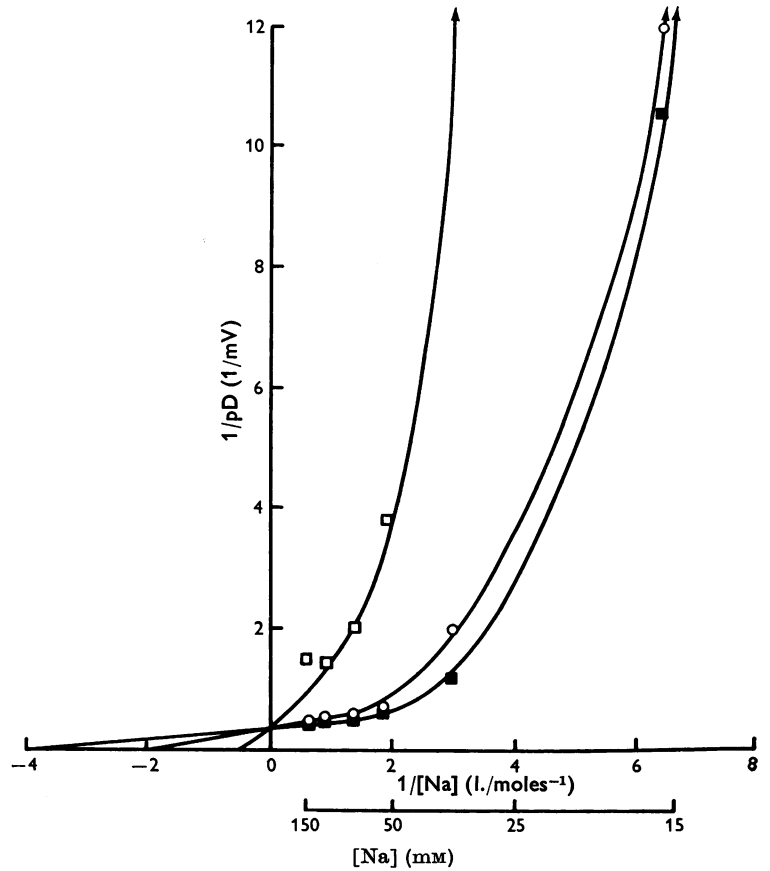

Fig. 5. Effect of sodium concentration on the glucose-evoked potentials of goldfish intestines taken from fish acclimatized to different temperatures. Each point is the mean of 3–5 values. Acclimatization temperature: □, 30°; ○, 13°; ■, 3° C.

Effect of sodium concentration on the steady-state potentials of goldfish intestines taken from fish acclimatized to different temperatures. Fish were acclimatized to 3, 13 and 30° C, the anterior intestines were everted and the steady-state potentials recorded, with and without glucose, at an incubation temperature of 25° C. The results, shown in Fig. 6, have been pooled for the 3 and 13° intestines because no differences could be seen at these two temperatures of acclimatization. In these cases the steady-state

potentials increased as the sodium concentration was reduced from 140 to 50 mM. This increase was more noticeable in the absence of glucose. At lower concentrations of sodium the steady-state potentials fell and became lower in the presence of glucose than in its absence. This is the same situation as that found previously with goldfish taken from water at room temperature (Smith, 1965). The intestines of fish acclimatized to 30° C maintained lower steady-state potentials than those from fish acclimatized to 3 or 13° C and the potential remained higher in the absence of glucose even at high concentrations of sodium. Reducing the

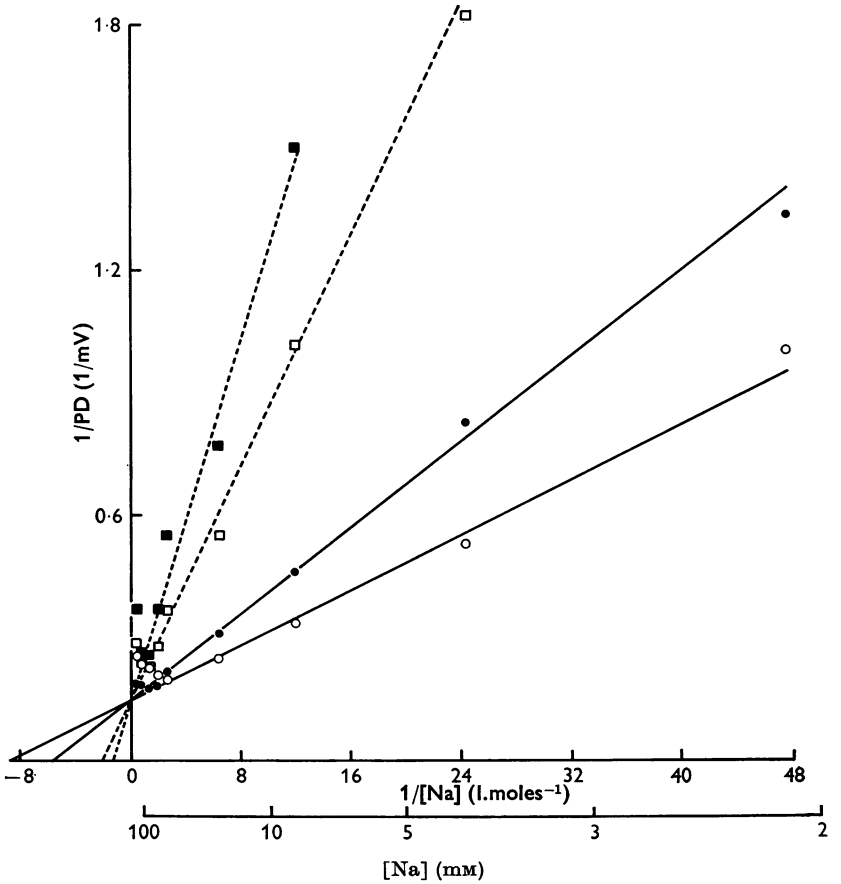

Fig. 6. Effects of sodium concentration on the steady-state transmural potentials of everted intestinal sacs taken from goldfish acclimatized to different temperatures. Open symbols, no glucose; closed symbols, 27 mM glucose present. The dotted lines are drawn from values obtained with 30° intestines (mean of three determinations) and the unbroken lines from values obtained using 3 and 16° C intestines (mean of eight determinations).

sodium concentration caused an increase in the steady-state potentials, as it did in the intestines of fish acclimatized to lower temperatures, but glucose remained inhibitory and the potentials began to fall when the sodium concentration was reduced below 70 mM. The fall in steady-state potentials was more dependent on the external concentration of sodium than in cold acclimatized fish and values could not be determined accurately below 8 mM sodium.

Effect of glucose concentration on the glucose-evoked potential of intestines taken from goldfish acclimatized to different temperatures. Glucose-evoked potentials were measured across everted preparations of goldfish intestines at a number of different glucose concentrations. The goldfish

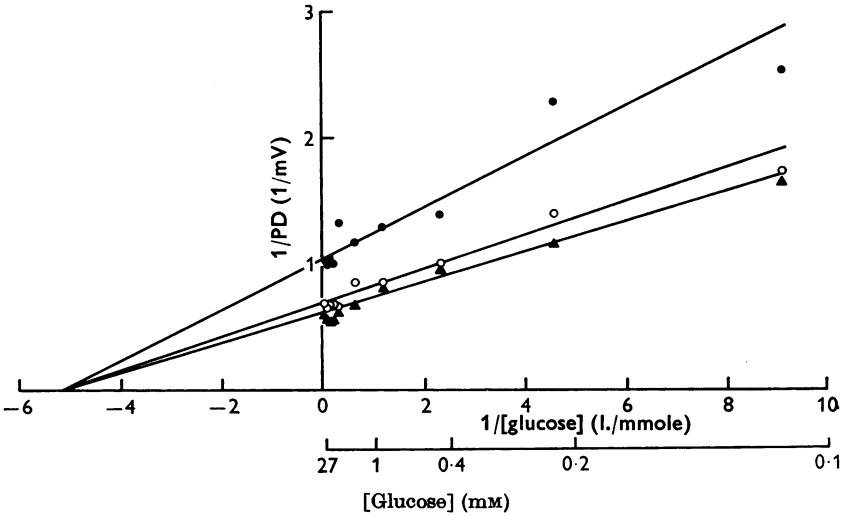

Fig. 7. Effect of glucose concentration on the glucose-evoked potentials of goldfish intestines taken from fish acclimatized to different temperatures. Acclimatization temperature: ●, 30°; ▲, 13°; ○, 3° C. Each point is the mean of 3–5 values.

had been acclimatized previously to one of three temperatures (3, 13 or 30° C). The results, summarized in Fig. 7, show that for the glucose concentration range 27–2 mM there is little change in the glucose-evoked potential but that over the range 2–0·1 mM the potential falls in proportion to the concentration of glucose used. These events are shown not to be dependent on the temperature of acclimatization, and though there is some scatter of points it is possible to draw straight lines through any set of values so that all three lines meet on the x-axis. There are therefore no grounds for assuming that temperature acclimatization can change the apparent affinity of glucose for the sodium–glucose interaction. This

affinity appears to be quite high, 0·2 mM glucose is sufficient to elicit a half-maximal response. The 30° intestines show consistently smaller glucose-evoked potentials than intestines taken from fish acclimatized to lower temperatures.

It has been shown in Fig. 1 that the steady-state potential with glucose present, recorded across an intestine taken from a fish adapted to 30° C, is lower than when glucose is absent. Figure 8 shows how this relation changes when the glucose concentration in the medium bathing the mucosa is lowered. The temperature of incubation was 25° C and in the

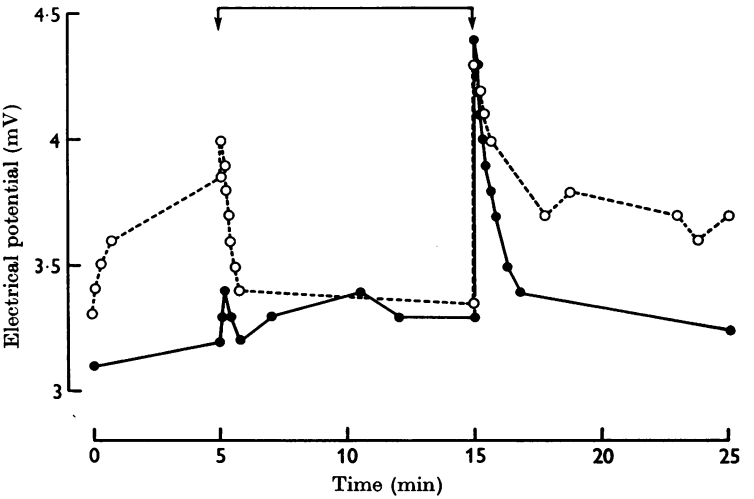

Fig. 8. Effect of changing glucose concentrations on steady-state potentials of a goldfish intestine taken from a fish acclimatized to 30° C. The everted sac was incubated at 25° C in bicarbonate saline containing 27 mM (●) or 16 mM (○) glucose. The sac was placed in medium with no glucose for the time period enclosed by the arrows.

presence of 27 mM glucose the steady-state potential is slightly lower than when there is no glucose. On placing the sac into medium containing 16 mM glucose, the transmural potential increased rapidly to a new stable level and now the transmural potential fell when the sac was transferred to glucose-free medium. In spite of this large change in steady-state potential, the glucose-evoked potential remained unaffected.

DISCUSSION

Many changes can be shown to take place in goldfish after periods of acclimatization to different temperatures. Tissues taken from cold-acclimatized goldfish use more oxygen than from heat-acclimatized fish when measured at some intermediate temperature (Freeman, 1950;

Ekberg, 1958) and there are changes in the proportion of unsaturated to saturated fats and cholesterol to phospholipids (Hoar & Cottle, 1952). Also the total body water of the goldfish increases as an acute response to cold (Meyer, Westfall & Platner, 1956) but decreases with long exposure (Hoar & Cottle, 1952). The implications are that changes in oxidative metabolism, cell membrane permeability and osmoregulation are all part of the phenomenon of temperature acclimatization, but the evidence is in each case indirect.

Experiments reported here for the goldfish intestine again show changes which are dependent on environmental temperature and in this case there is internal evidence to show how these changes might serve to regulate sodium transport. At any acclimatization temperature in the range 8 to 30° C the rate of sodium transport, as measured by the steady-state potential, remains constant when recorded at the swimming temperature of the fish. There is however a general reduction in transmural potential in intestines taken from fish acclimatized to 3° C. This partial failure in acclimatization is one indication that the fish may now be susceptible to temperature changes in its environment and the low lethal temperature for goldfish is in fact quite close at about 0° C (Fry, Brett & Clawson, 1942). One might predict a similar failure to regulate sodium transport at environmental temperatures near the high lethal temperature (40° C), but this was not tested in the present paper.

Sodium has to enter and leave the mucosa for net transport to occur and both mucosal membranes must be considered as potential sites for the thermal regulation of sodium transport. If we believe that entry from the lumen is essentially a passive process, sodium moving down its concentration gradient by diffusion through holes or pores in the luminal membrane, then it is easier to think that temperature acclimatization might take place in the basal membranes of the mucosa, where the sodium pump is thought to move sodium against its concentration gradient. The goldfish intestine appears histochemically to possess an unusually large proportion of a highly specific ATPase at this site in the mucosa (Hollands & Smith, 1964) and it may be that this enzyme has some regulatory function associated with temperature acclimatization. But other evidence strongly suggests that sodium movement across the luminal membrane involves a degree of interaction with that membrane, possibly by the transitory formation of a sodium-carrier complex (Smith, 1965) and temperature acclimatization of sodium transport may be explained by changes confined to the luminal membrane. In coming to this conclusion it has been assumed that the intracellular sodium concentration never rises to the point where the basal membrane sodium pump becomes the rate-limiting step to sodium transport. This assumption is generally accepted for other

epithelial tissues (Frazier, Dempsey & Leaf, 1962; Cereijido, Herrera, Flanigan & Curran, 1964).

The glucose–sodium interaction is thought to occur at the outside face of the luminal membrane (Crane, Miller & Bihler, 1961; Schultz & Zalusky, 1964). Results reported here show obvious changes in this interaction in fish acclimatized to different temperatures. The values for the glucose-evoked potential fell as the temperature of acclimatization rose and at high temperatures of acclimatization no glucose-evoked potential could be detected at low incubation temperatures (Fig. 2). The variability of results makes it difficult to be certain that changes in Q_{10} values (i.e. rotational changes) occurred as a result of acclimatization, but the translational effect was obvious. Prosser (1958) has listed the types of change in enzyme rate which can occur as a result of temperature acclimatization. From this it appears that the shifts described above are often seen in studies of temperature acclimatization where oxygen consumption is measured as it has been, for instance, in the carp (Suhrmann, 1955). Translational effects can be accounted for simply by changes in enzyme concentration but rotational effects imply a change in activation energy of the enzyme in question.

The apparent affinity of the carrier for sodium was much reduced at high acclimatization temperatures (Fig. 5) but that for glucose was not changed (Fig. 7). It is important to specify that these results were obtained at an incubation temperature of 25° C because here the glucose-evoked potential was still in the rapidly rising phase (with respect to incubation temperature) in the 30° intestines but in the slowly rising phase for the 3–13° intestines. This is another way of saying that the carrier can exist in either of two forms depending on the temperature of incubation and that temperature acclimatization can favour one form over the other. This forces one to consider that the carrier can alter its conformational structure.

There are some startling similarities between the sodium–glucose interaction with its carrier enzyme and the relation which exists between an allosteric protein and its substrate plus allosteric effector. The theory that an allosteric protein possesses at least two quite separate receptor sites—one which binds the substrate and one which binds the allosteric effector—was first formulated by Monod, Changeaux & Jacob (1963) to provide a comprehensive explanation for a wide variety of related facts. This theory states that the allosteric effector acts solely by altering the molecular configuration of the enzyme to modify the substrate binding site. Imagine that sodium is the 'substrate' for the carrier enzyme (the allosteric protein) and that glucose is the allosteric effector. Then compare their behaviour to that of allosteric enzymes and effectors.

(1) Allosteric effectors bear no steric resemblance to the substrate and this distinguishes their action from that of a secondary substrate or substrate analogue. Glucose fulfils this requirement.

(2) An allosteric effector does not take part in the enzyme action; it acts solely by changing the conformational structure of the enzyme. Glucose increases the transport of sodium in the goldfish rectum where its own transport is small. This anomaly disappears if glucose is acting as an allosteric effector.

(3) Analogues of allosteric effectors either behave like the natural allosteric effector or displace it to block subsequent conformational changes. Analogues of glucose (galactose, 3-*O*-methyl glucose, α-D-methyl glucose; Schultz & Zalusky, 1963) mimic glucose in stimulating the sodium-carrier reaction, and phlorrhizin, which has some structural similarities to glucose, blocks the reaction (Smith, 1965).

(4) Allosteric effectors of different structure can separately activate the same allosteric enzyme by binding to different stereospecific allosteric receptor sites. Some sugars act to promote sodium transport (Schultz & Zalusky, 1963) and so do amino acids (Schultz & Zalusky, 1965) and both appear to act on the same carrier enzyme, possibly in the way described above.

(5) The ability of an allosteric effector to cause conformational changes is very easily modified by many procedures, including gentle heating. The ability of glucose to affect the sodium carrier is also very sensitive to temperature changes and sodium transport quickly fails in 12° intestines maintained at 35° C (Smith, 1964).

(6) Structural changes of the allosteric protein necessary to convert the enzyme from a desensitized to a sensitized state are very small and the protein will bind both substrate and effector in either state. The intestinal carrier will also bind sodium and glucose in the fully sensitized (at 25° in fish acclimatized to 3–13° C) or partly sensitized (at 25° in fish acclimatized to 30° C) state.

7. At substrate concentrations below half saturation of the allosteric enzyme, the reaction velocity increases faster than the substrate concentration in cases where the active site can bind more than one substrate molecule. The same is true for sodium and its carrier ($1/PD$) representing the rate of sodium transfer).

8. The abnormal kinetic behaviour of a sensitized enzyme is made more normal by desensitization. The kinetics of the sodium–glucose interaction at reduced sodium concentrations is also more normal for the partly sensitized carrier (measured at 25° in fish acclimatized to 30° C) than for the fully sensitized carrier (measured at 25° in fish acclimatized to 3–13° C).

Monod *et al.* (1963) point to the biological significance of allosteric enzymes in controlling the adaptation of a cell to its environment and it seems probable that the goldfish intestine can use glucose as a signal to alter sodium transport under different environmental conditions. Though there are many parallels between the observed behaviour of allosteric changes and the sodium–glucose carrier, this is of course not proof that intestinal sodium transport is partly under the control of allosteric enzymes, and similarities reported here must now be tested further.

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