Puromycin Inhibition of Changes in Glucose-Evoked Potential Recorded in Goldfish Intestine after Periods of Temperature Acclimatization

The goldfish intestine resembles that of mammals in its ability to maintain a transmural electrical potential which is dependent partly upon the presence of glucose[1]. Addition of glucose to medium bathing the mucosa causes an immediate increase in the transmural potential and it is this 'glucose-evoked' potential which has been taken to represent the initial combination of glucose and sodium with a carrier in the brush border membrane of goldfish mucosal cells[2]. Similar interactions are thought to occur in the hamster and rabbit small intestine[3,4]. Acclimatization of the goldfish to a high environmental temperature reduces the glucose-evoked potential measured at different incubation temperatures[5], and it has been suggested that this change is caused by the synthesis of carrier molecules that differ in some qualitative way from the original ones[6]. The present experiments were designed to test this suggestion.

Goldfish, fully acclimatized to 8 °C, were decapitated and the anterior intestine removed, everted and tied to a polythene cannula. The other end of the intestine was tied with thread to form an open-ended sac and 0.1 ml of bicarbonate saline[7] placed inside. The cannulated sac was then immersed in oxygenated bicarbonate saline at room temperature, and the transmural potential was recorded with a Vibron electrometer through agar bridges and calomel electrodes. The output voltage from the electrometer was led to a 'Xactrol' pen recorder to obtain a permanent record of changes in potential. After 15–30 min at room temperature, during which time the transmural potential increased to a steady value, the sac, electrodes and oxygenator were transferred to an apparatus containing fresh bicarbonate saline equilibrated at a number of different temperatures (for full description see SMITH[5]). Steady transmural potentials were recorded, with and without glucose (5 mg/ml) in contact with the mucosa, and the glucose-evoked potentials were measured at ten different incubation temperatures.

The glucose-evoked potential increased rapidly as the incubation temperature was raised from 7.5–13 °C and then more slowly as the incubation temperature was further increased from 13–30 °C (Figure 1). The lower range of incubation temperatures, where the glucose-evoked potentials were shown to be highly temperature dependent, has been called *phase 1* and the higher range of incubation temperature *phase 2*. The energy of activation for the combination of sodium with its carrier, assuming this to be an enzymic event and that the glucose-evoked potential is a measure of the initial rate of reaction of sodium with the carrier, was 30,700 cals during phase 1 and 14,200 cals during phase 2 (mean of 19 experiments). The intraperitoneal injection of 0.05 ml, 0.9% w/v NaCl, 21 h before killing the fish, did not change this pattern. Neither did the injection of puromycin (Nutritional Biochemicals Co.) dissolved in 0.9% w/v NaCl solution (7.9–11.9 µg/g goldfish) although in this case the glucose-evoked potentials were smaller than the corresponding control values. This amount of puromycin produced no noticeable toxic effects in the goldfish up to 21 h after the injection. Changing the environmental temperature of goldfish from 8–25 °C reduced the glucose-evoked potentials measured 20 h later. The range of phase 1 was now 7.5–23 °C instead of 7.5–13 °C (Figure 2). Intraperitoneal injection of puromycin, 8.2–11.4 µg/g goldfish, 1 h before the environmental temperature was raised, stopped this extension of phase 1, the temperature at which phase 1 changed to phase 2 remaining at about 13 °C. The glucose-evoked potentials, seen after injection of puromycin, were small compared to those obtained with intestines from control non-injected fish acclimatized to 8 °C, and

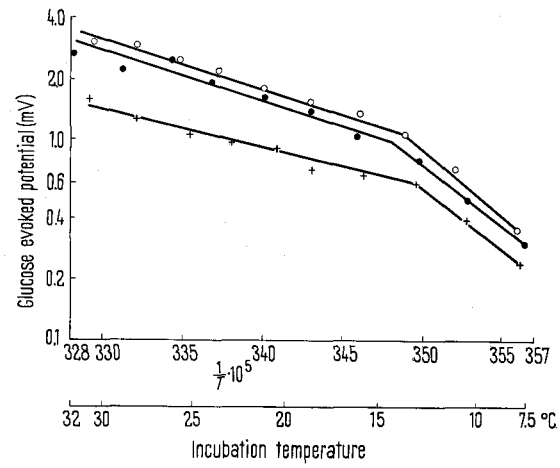

Fig. 1. Glucose-evoked potentials of goldfish intestine measured at different incubation temperatures. The environmental temperature of the goldfish was maintained at 8 °C for at least 14 days prior to the experiment. —o—, control experiments (19 determinations); —●—, 21 h after the i.p. injection of 0.05 ml, 0.9% w/v NaCl solution (1 experiment); —+—, 21 h after the i.p. injection of 0.05 ml, 0.9% w/v NaCl solution containing 500 µg puromycin (5 experiments). $1/T$ is the reciprocal of the incubation temperature measured in degrees absolute.

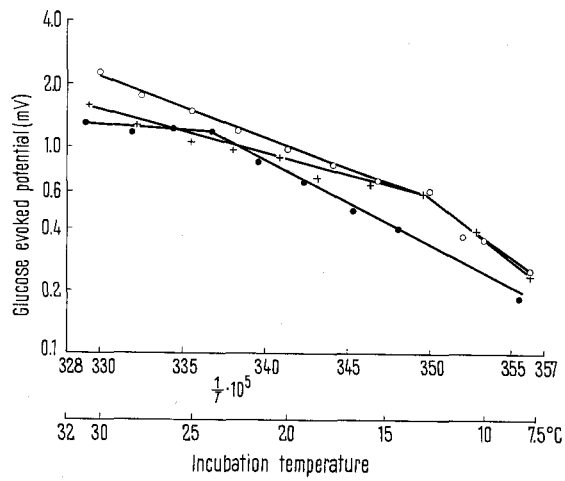

Fig. 2. Glucose-evoked potentials of goldfish intestine measured at different incubation temperatures. Goldfish were fully acclimatized to 8 °C and then either used 20 h after changing the environmental temperature to 25 °C (—●—, 4 experiments); 21 h after the injection of 500 µg puromycin, the environmental temperature being maintained at 8 °C (—+—, 5 experiments); 21 h after the injection of 500 µg puromycin, the first h after injection being spent in water at 8 °C and the following 20 h at 25 °C (—o—, 5 experiments).

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similar to previous values determined on intestines from fish acclimatized to 8 °C, taken 21 h after injection of the same quantity of puromycin (Figure 2).

Another way of observing the effect of temperature acclimatization on electrical parameters of the goldfish intestine is to measure the incubation temperature at which glucose first causes a permanent increase in the steady transmural potential. This temperature has been found to be approximately equal to that at which glucose-evoked potentials change from a phase 1 to a phase 2 type of behaviour[6]. This temperature is not changed by injection of puromycin when the environmental temperature is kept constant at 8 °C (12.5 °C for control intestines; 13.1 °C when injected 21 h previously with puromycin). However puromycin does retard the change in this temperature which occurs when fish acclimatized to 8 °C are subjected to 25 °C for 20 h (26.6 °C for goldfish at 25 °C for 20 h; 18.4 °C for similar fish injected with puromycin 1 h before changing the environmental temperature). These values are each the mean of five determinations and the difference between the two populations is significant ($t = 3.62$; $P < 0.01$).

It is therefore suggested that a carrier for sodium ions, activated by glucose and thought to be situated in the luminal membrane of the goldfish mucosa, is replaced by a different carrier under the stimulus of a changed body temperature and that, since puromycin stops this substitution, the carrier is either a protein or else dependent on the presence of protein molecules for its normal operation.

Résumé. Le potentiel transmural de l'intestin du poisson rouge, incubé in vitro, dépend partiellement de la présence de la glucose. Ce potentiel, dû à la glucose, est déterminé par la température d'acclimatation, et se réduit dans la mesure que cette température augmente. Cet aspect de l'acclimatation est complet 20 h après qu'on a élevé la température du milieu du poisson, et le puromycin entrave ces variations. Ces résultats suggèrent l'idée que les variations dans les propriétés des membranes de l'epithélium intestinal impliquent la synthèse des molécules de protéine.

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Effect of Thymectomy on Immune Response in Adult Rats

Miller[1] observed in 1962 that total body irradiation considerably decreased the immune response and increased the tolerance of skin grafts in thymectomized young adult mice. He concluded that the thymus was immunologically important, even in adult age. Many authors supported these results, but there were a lot of contradictory data[2–5]. We observed in earlier experiments[6] a significant rise of the immune response in rats a long time after they had been thymectomized. It was in view of such contradictory results that the present experiments were designed with the object of ascertaining the effect of age and that of time elapsed since thymectomy.

62 rats of the Wistar strain were used. They belonged to three age classes: 4, 9 and 13 months. The 4-month group contained only animals that had been thymectomized a week before the first immunization, while a part of the animals in the other two groups had also been thymectomized a week before, another part 6 months and still another part 10 months before the beginning of immunization. Intact animals of corresponding ages served as controls. The plan of the experiments is tabulated in Table I. (See the experimental and mathematical methods in [6].)

Mathematical-statistical results (Table II): As regards primary response, the difference between controls and long thymectomized animals was somewhat above the level of significance in the 9-month group, and somewhat below significance in the 13-month group. Since the deviation of about one tube in favour of thymectomized animals, observed in both groups, is in good harmony with earlier observations[6], immune response promoting tendency can be regarded as proved for both groups.

As regards secondary response, no significant deviation was registered in any group. This situation was found unchanged 2 weeks after the irradiation without renewed antigenic stimulation.

As regards tertiary immune response, a significant decrease was registered in comparison to the controls in the freshly thymectomized members of the 4- and 13-month groups, a result in good agreement with those

Table I. Plan of the experiments

Time in weeks	Experiments
0	Immunization with sheep red cells (SRC) intravenously $100 \cdot 10^6$/100 g body weight
1	Hemagglutination[a]
2	–
3	Immunization with SRC $150 \cdot 10^6$/100 g
4	Hemagglutination[b]
5	350 r total body X-ray irradiation
6	–
7	Hemagglutination[c]
8	Immunization with SRC $100 \cdot 10^6$/100 g
9	Hemagglutination[d]
10	Hemagglutination[e]

The superior letters indicate the identical groups in Table II.

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