Methionine Transfer Across Goldfish Intestine Acclimatized to Different Temperatures

The intestinal mucosa of the goldfish synthesizes protein in vivo at a rate which depends on the body temperature of the fish[1]. The amino acid composition of the synthesized protein also changes; the amount of valine decreases and the amount of methionine increases as the environmental temperature is raised[2]. The transport of valine across the mucosa can also be altered by acclimatization to different temperatures[3] but nothing is known about the transport of methionine under similar conditions. The present experiments measure the temperature-dependence of methionine transport to determine whether there is any connection between the transfer of these 2 amino acids and their incorporation into mucosal protein.

Goldfish weighing about 50 g were kept in aerated water at 8°, 15°, 20°, 25° or 30 °C for 2–3 weeks before use. Fish in cold water ate less than those in warm water and food was given to all in a slight excess up to the time of the experiment. Fish were killed by decapitation and the anterior intestine everted on a polythene covered metal rod to form 2 sacs which were both filled with 100 μl of bicarbonate saline[4] containing 5.5 mM glucose and 10 mM methionine. The everted sacs were suspended from open-ended cannulae in 15 ml of the same methionine-containing bicarbonate saline gassed with a mixture of 95% O_2 + 5% CO_2. One sac was incubated at 30 °C and the other at a temperature equal to the previous environmental temperature of the fish. The sacs were removed after incubation for 2 h and the serosal solutions transferred with the aid of a syringe into weighed containers. The volume of fluid recovered was estimated by weight. The intestines were blotted and then weighed rapidly on a sensitive torsion balance (± 1 mg). Methionine was estimated colorimetrically[5].

Figure 1 shows the results obtained. Each value is the mean of 6 determinations, 3 using the proximal part and 3 the terminal part of the anterior intestine. The serosal transfer of methionine (Figure 1, left side), measured at 30 °C, fell as the acclimatization temperature was raised, showing that some regulation of methionine transfer had taken place. But when the incubation temperature was adjusted to be the same as the environmental temperature of the fish, the transfer of methionine rose with in-increasing temperature, showing that the regulatory mechanism was not compensating fully for the rise in environmental temperature. The ability of goldfish intestine to concentrate methionine at its serosal surface when incubated at 30 °C showed no dependence on the previous acclimatization temperature (Figure 1, right side). The corresponding data for valine, calculated from previous work[3], show a final serosal concentration of 22 mM for 8 °C-acclimatized intestines and 15 mM for 25 °C-acclimatized intestines, when both are incubated in 10 mM-valine at 25 °C. This suggests, but does not prove conclusively, that the ability of the goldfish intestine to concentrate valine is being changed by acclimatization to a high temperature and that in this respect it differs from methionine.

Fluid transfer has been plotted against the serosal transfer of methionine in Figure 2. Both fluid and methionine transfer were measured at 30 °C using intestines taken from fish acclimatized to different temperatures. The transfer of fluid was reduced fivefold and the methionine transfer twofold for a 22 °C shift in the acclimatization temperature. The regulation of methionine transfer seems therefore to be concerned primarily with the regulation of fluid (and therefore sodium) transfer.

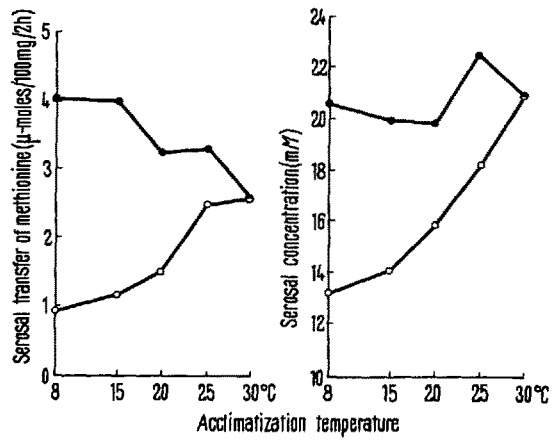

Fig. 1. The effect of temperature acclimatization on various aspects of methionine transfer across the goldfish intestine. Everted sacs of goldfish intestine were incubated for 2 h in bicarbonate saline containing 5.5 mM D-glucose and 10 mM L-methionine, gassed with 95% O_2 + 5% CO_2. 100 μl of the same solution was placed in each sac at the start of incubation. —●—●—, serosal transfer or serosal concentration of methionine measured at 30 °C; —o—o—, serosal transfer or serosal concentration of methionine measured at incubation temperatures equal to the previous environmental temperatures of the fish. Each point gives the mean of 6 determinations.

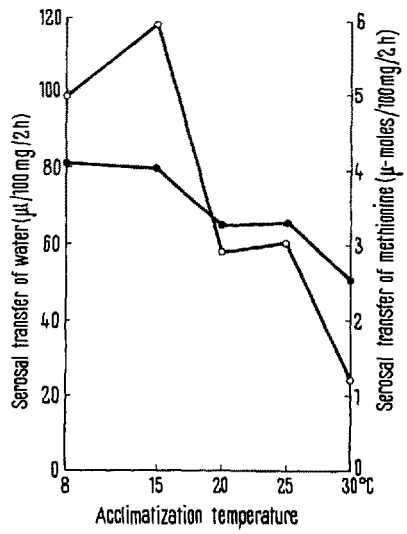

Fig. 2. Effect of temperature acclimatization on fluid transfer across the goldfish intestine. Everted sacs of goldfish anterior intestine were incubated as described in the legend to Figure 1, at a constant temperature of 30 °C. ●—●—, serosal transfer of methionine; o—o—, serosal transfer of fluid. Each point gives the mean of 6 determinations.

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When the present results on methionine transfer are compared with previous estimations of methionine, free in the intestinal mucosa and in the extracted protein[2], it can be seen that all 3 levels increase by about the same proportion for the same shift in acclimatization temperature, implying that the ability of the mucosal cell to concentrate methionine determines its own incorporation into protein. Methionine is known to play a critical part in the aggregation of polysomes[6] and in the initiation of peptide synthesis[7] and it may act in the goldfish intestinal mucosa, in opposition to valine, as a pacemaker for other processes involved in the metabolic regulation of cells exposed to different temperatures. To reach this conclusion it has been assumed that the amounts of methionine and valine reaching the outside of the mucosal cell are always sufficiently constant to be ignored as a possible factor influencing intracellular events. This situation applies in vitro but may not be true in the intact fish where the amount of food consumed varies with the environmental temperature of the fish[8].

Zusammenfassung. Es konnte gezeigt werden, dass der Methionintransport im in-vitro-Dünndarmpräparat des Goldfischs temperaturabhängig ist: 1. Temperatur, bei welcher der Fisch akklimatisiert wurde, 2. Temperatur, bei welcher der Darm inkubiert wurde. Die Endkonzentration von Methionin auf der Serosaseite hängt ebenfalls von der Inkubationstemperatur, nicht aber von der Akklimatisationstemperatur ab. Der Flüssigkeitstransport, gemessen bei 30 °C, war um das Fünffache geringer, sobald die Akklimatisationstemperatur von 8 auf 30 °C stieg.

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Studies on Chemotaxis. VII. Cytotaxins in Rabbit Serum

Using Boyden's technique for measuring chemotaxis, the action of substances found to be chemotactic in vivo can be analysed in vitro[1]. It was shown that antigen-antibody complexes are not chemotactic per se, but exert their chemotactic effect by formation of chemotactic mediator(s) (cytotaxins)[2] in the serum. Similarly the chemotactic effect of other agents such as endotoxins, bacteria, tuberculin, glycogen or heat aggregated γ-globulins is mediated by cytotaxins formed in fresh serum[2]. The cytotaxins generated on incubation with antigen-antibody complexes are specific for polymorphs[3], and appear to differ from cytotaxins from other sources[4]. Evidence has been presented that these polymorph cytotaxins are identical with a complex consisting of the complement components C'5, 6, and 7[5]. On the other hand, it has been found that fixation of hemolytic complement and formation of cytotaxins may occur independently[6]. In the present study, further data on the properties of serum cytotaxins are reported which show that these findings are not mutually exclusive.

The thermostability of polymorph cytotaxin(s) has been tested by incubating aggregated bovine γ-globulin treated rabbit serum (aBGG) at various temperatures for 30 min. The chemotactic activity after heating was then calculated from a standard curve established in the same experiment with several concentrations of chemotactic serum heated to 56 °C. The curve given in Figure 1 is derived from the mean values of 3 experiments. The data show a pronounced loss of activity between 65 and 80 °C. A low but definite activity can still be observed at higher temperatures up to 100 °C. Whether this remaining activity is due to partial inactivation of a single cytotaxin, or to the presence of another cytotaxin differing in its thermostability, needs further investigation.

Evidence for the existence of more than one polymorph cytotaxin in rabbit serum has indeed been obtained in the following experiments. aBGG or antigen-antibody treated rabbit serum was separated on a Sephadex G-200 column using a 0.1 *M Tris*-HCl/1.0 *M* NaCl buffer pH 8.0.

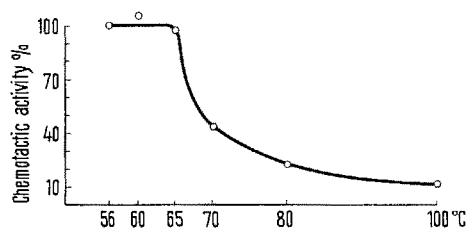

Fig. 1. Effect of heating on polymorph cytotaxins in rabbit serum.

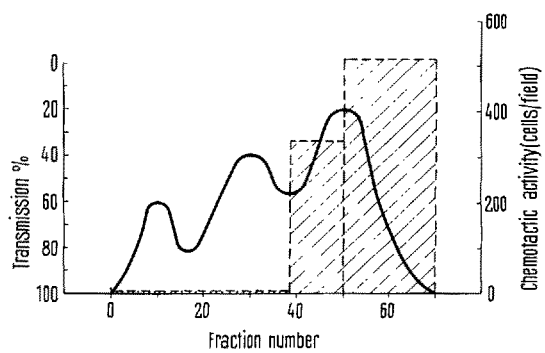

Fig. 2. Chemotactic activity of serum fractions eluted from Sephadex G-200 (eluant: 0.1 *M Tris*-HCl + 1 *M* NaCl) on polymorphonuclear leucocytes.

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