tured for 6 days, and in 13-day-old explants cultured for 4 days. Results with control ovaries and testis, as well as with parabiotic pairs cultured with media with gonadotrophins, showed no difference to the above-mentioned results.

The fact that germ cells in embryonic chick ovaries are capable of initiating meiotic prophase while cultured in vitro agrees with results obtained on other avian[11,12] and mammalian[13] embryos. The earliest indication of gonadotrophic action in chick embryos has been found by FUGO[14] on the 13th day of incubation. It may be admitted that very minute amounts of gonadotrophins may exist in 9-day-old chick embryo extracts, but culture media containing them are considered for practical purposes as non-hormonal. Cultured in these conditions, the time needed by germ cells in ovarian tissue to reach meiotic prophase was similar to that needed by germ cells in ovaries of the same age developed in vivo (HUGHES[1]). All these facts appear to indicate that no extragonadal hormonal action is necessary for this process to take place. This supposition also receives support from the fact that the addition of great amounts of gonadotrophins to the media did not alter germ cell behaviour.

As hormones produced by gonads in organ culture diffuse freely[15], it can be accepted that germ cells in both ovarian and testicular tissues cultured together were subjected to similar hormonal stimulation, although differences in concentration may have existed. Differences in the behaviour of germ cells in both types of tissues are then difficult to explain on a hormonal basis.

It may be suggested that the action of cells in direct contact with germ cells could explain this different behaviour. OHNO and SMITH[16] hold that correct differentiation of mammalian oocytes depends on the presence of a sufficient number of follicular cells surrounding them during the embryonic period. The inability of germ cells in testicular tissue to initiate meiosis in our cultures would then perhaps be due to lack of adequate differentiation of supporting cells surrounding them[17].

Resumen. Ovarios y testículos de embrión de pollo fueron cultivados en aposición. En los ovarios las células germinales iniciaron la profase meiótica mientras que en los testículos aquellas permanecieron indiferenciadas. El agregado de hormonas al medio no modificó la situación.

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Temperature Acclimatization and Protein Synthesis in the Goldfish Mucosa

Everted sacs of goldfish intestine maintain transmural potentials which depend on the previous acclimatization temperature of the fish[1]; changes in potential which occur as cold-adapted fish acclimatize to warm water can be inhibited by the previous injection of puromycin[2]. The relative permeability of mucosal cell membranes to ions appears therefore, at least during periods of acclimatization, to be controlled through protein synthesis in the mucosal cell. The present experiments were designed to measure the rate of protein synthesis in the mucosae of 8 °C-acclimatized goldfish as they adapted to 25 °C and to determine the effect of actinomycin D, a specific inhibitor of DNA directed M-RNA synthesis, on protein synthesis during this period of acclimatization.

Goldfish weighing about 50 g were fed daily and kept for 2–3 weeks in aerated water at a temperature of 8 °C. In one series of experiments 50 μc of [G-^{3}H] DL-valine or [G-^{3}H] DL-threonine (from the Radiochemical Centre, Amersham) were injected intraperitoneally into fish at 8 °C or at different times after the transfer of 8 °C-acclimatized fish to water maintained at 25 °C and in some cases actinomycin D was injected intraperitoneally (1 μg/g body weight) at the time of transfer. For comparison, fish acclimatized to 8 or 25 °C for 2–3 weeks were given the tritiated amino acid and actinomycin D at the same time. All fish were decapitated 30 min after the injection of amino acid. The anterior intestine was removed, rinsed with physiological saline and the mucosa squeezed out and homogenized in 1 ml of ice-cold saline. 1 ml of 24% w/v trichloroacetic acid was added and the suspension again homogenized. These operations were completed within 5 min of killing the fish. The protein suspension was heated at 90 °C for 1 h, centrifuged for 5 min at 3000 rpm, and the precipitate washed twice with 5 ml of 12% w/v trichloroacetic acid. Lipids were extracted with 5 ml of acidified chloroform methanol (1:1, v/v), the suspension being left for 20 min to aid extraction. The extraction was repeated with a 2:1 mixture of acidified chloroform methanol and the protein then dried with two 5 ml washings of sodium-dried ether. Weighed samples of protein were dissolved in hyamine hydroxide (Nuclear Enterprises Limited, Edinburgh) and tritium estimated with a Tri-Carb liquid scintillation spectrometer (Packard Instrument Company Inc.). It was assumed that the rate of tritium incorporation represented the true rate of protein synthesis. This will be true provided the concentrations of amino acids at the site of protein synthesis remain constant throughout acclimatization.

Figure 1 shows the rate of incorporation of tritium into mucosal protein at different times during acclimatization to 25 °C. The rate of protein synthesis in 8 °C-acclimatized

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fish is about $^1/_3$ that measured 30–60 min after raising the environmental temperature to 25 °C. This immediate, actinomycin D-resistant, increase in protein synthesis could have been caused by activation of normally quiescent ribosomes[3]. 30 h after raising the environmental temperature, the rate of protein synthesis was still somewhat higher than in 8 °C-acclimatized fish and not different from fish kept at 25 °C for 2–3 weeks. Acclimatization from 8–25 °C appears to be complete within 30 h of

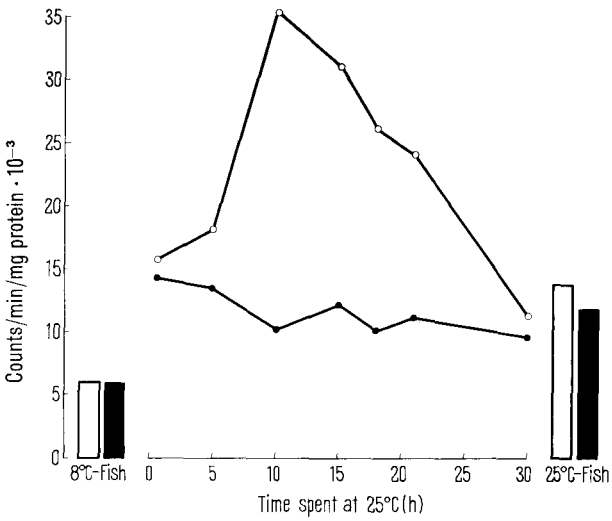

Fig. 1. Protein synthesis in goldfish mucosa measured during temperature acclimatization. Tritiated threonine or valine was injected i.p. (50 μc in 0.05 ml of 0.9% w/v NaCl) and the mucosa extracted 30 min later. Each point is the mean of 4 determinations, 2 with threonine and 2 with valine. Open histograms and circles show control fish and closed histograms and circles, fish injected with actinomycin D (1 μg/g fish, 1 mg/ml solution in 0.9% w/v NaCl). The actinomycin D was injected into fully acclimatized fish together with the tritiated amino acid or at the time of transfer of 8 °C-acclimatized fish to 25 °C.

changing the environmental temperature (see Figure 1). There was an additional increase in protein synthesis in control fish when measured 10 h after raising the environmental temperature which was not seen if actinomycin D had been injected at the time of transfer. The difference between these 2 values was significant at the 10% level of probability ($t = 2.15$, $P < 0.10 > 0.05$). At times greater than 10 h the control synthesis fell rapidly, becoming the same as that measured in fish injected with actinomycin D. Conclusions drawn from the shape of these curves must be tentative only because of the variation between individual fish. This is illustrated in Figure 2. Here the curves for untreated and actinomycin D treated fish are analysed over the time period 10–30 h after raising the environmental temperature. The regression for untreated fish with standard error was -0.0263 ± 0.0131 which is very nearly significant at the 5% level of probability. The regression for fish injected with actinomycin D was 0.0053 ± 0.0131 which is not significantly different from 0. The regression for untreated fish differed from the actinomycin D treated fish at the 10% but not at the 5% level of probability. No difference could be detected between valine or threonine, used to measure protein synthesis. The free amino acid pools in the muscle of some marine fish are known to be variable[4] and if this were true for the goldfish, it would partly explain this variation.

There is nevertheless fair evidence to suggest that acclimatization of the goldfish mucosa to a raised temperature involves not only an immediate increase in the rate of protein synthesis but also a delayed increase occurring after a 10 h lag period. It is possible that new M-RNA molecules have to be synthesized to induce this second surge of protein synthesis since actinomycin D reduces it. The time course of temperature acclimatization appears to be sequential with a 10 h peak of protein synthesis preceding a 15–20 h change in membrane characteristics of the mucosal cell[5]. The exact relation between these 2 events requires further study.[6]

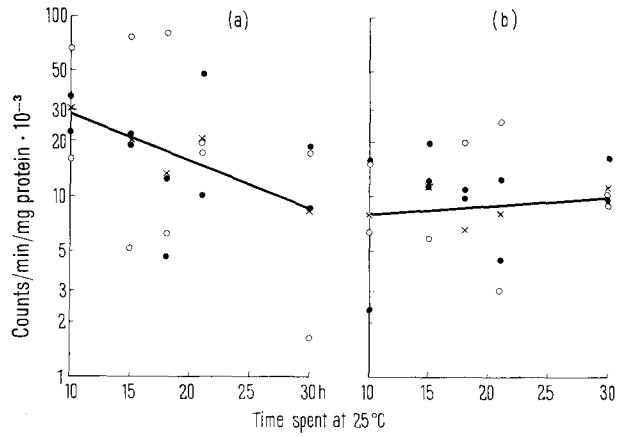

Fig. 2. Significance of changes in protein synthesis in goldfish mucosa, induced by changing the environmental temperature, measured in the absence (a) or presence (b) of actinomycin D. –o–, incorporation of tritiated threonine; –●–, incorporation of tritiated valine; –x–, mean values. Lines have been calculated as best fits to all experimental points. Fish were acclimatized to 8 °C for 2–3 weeks and then transferred to 25 °C for 10–30 h. The injection of tritiated amino acids and of actinomycin D was as described for Figure 1.

Resumen. La síntesis de proteinas en la mucosa del pez dorado aclimatado a 8 °C, aumenta inmediatamente cuando la temperatura del baño aumenta a 25 °C. Un nuevo aumento en la síntesis de proteinas se observa después de 10 h. Este último aumento es inhibido por actinomicina D. La reorganización en la síntesis de proteinas parece completarse dentro de las 30 h siguientes al aumento de temperatura. A pesar de que se observan variaciones grandes en diferentes peces la evidencia sugiere que al menos parte del fenómeno de aclimatación en la mucosa del pez dorado está relacionado con la síntesis del RNA mensajero.

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