Germ Cell Differentiation in Embryonic Gonads Cultured in Heterosexual Parabiotic Pairs

In bird and mammalian embryos, oogonia initiate meiotic prophase and stop it after pachytene stage has been reached[1,2]. Spermatogonia, on the contrary, do not start meiosis until sexual maturity, in the post-natal period. Experiments on sex inversion made on bird[3,4] and mammalian[5] embryos appear to indicate that difference in evolution of germ cells in both sexes does not depend on their genetical constitution but on the action of surrounding gonadal tissues. The fact that in bird embryos both ovaries and testis secrete hormones[6-8] suggested the possibility that these hormones may be involved in germ cell differentiation. It has been demonstrated that the growth of embryonic gonads in apposition with gonads of the opposite sex[9], or with other target organs[10], is an excellent procedure in order to study the action of embryonic gonadal hormones. This same technique has now been used by us to establish the possible action of these hormones on germ cell differentiation.

Several experiments were made as described in the Table. Cultures were made following the technique of WOLFF and HAFFEN[9]. The medium was a slight modification of the standard one used by those authors:

E.E. (8- to 9-day-old embryos)	25%
Eagle's basal medium	25%
Agar solution (1% in Hank's)	50%
Penicillin	10,000 U/ml

Whole gonads of 8-day-old embryos and pieces of gonads of 10- and 13-day-old embryos were cultured in several different combinations as explained in the Table. In all cases, White Leghorn chick embryos from the same source were used.

Explants were fixed after 4, 7 and 14 days of culture. In the last case the medium was renewed on the seventh day. After fixation with Bouin's fluid, explants were studied with routine histological techniques (H. and E.).

As shown in Figure 1 parabiotic pairs of gonads differentiated well and fused completely, although the limit between tissues of both origins was always identifiable. In some cultures testicular tissue was in contact with ovarian medulla (Figure 1), and in others with the cortex. Both isolated ovaries cultured as controls and ovarian tissue in parabiotic pairs showed the same evolution as ovaries developed in vivo: the germ cells contained in their cortex transformed into oocytes in different phases of meiotic prophase (Figure 2). In parabiotic pairs formed by 8-day-old gonads it was found that the germ cells in ovarian cortex had started meiotic prophase after 7 days of culture, while no meiotic prophase was seen in testicular tissue of the same cultures after 14 days of culture. The same difference was observed in 10-day-old gonads cul-

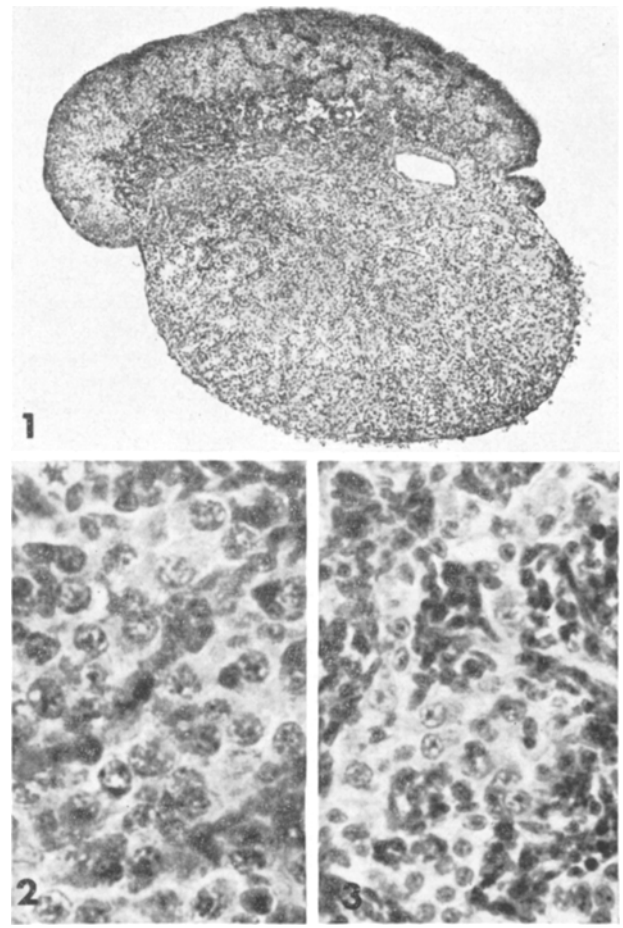

Fig. 1. Heterosexual parabiotic pair of 8-day-old gonads, cultured during 7 days. H. and E., × 90.

Fig. 2. Ovarian cortex from the same explant at higher magnification. H. and E., × 800.

Fig. 3. Testicular cords from the same explant. H. and E., × 700.

Series[a]	Hormone added to the medium (100 γ/ml)	Type of explant (days)	No. of explants[b]	Duration of culture (days)
1	–	♀ + ♂ 8	70	7
2	–	♀ + ♂ 8	65	14
3	–	♂ 10		
		♀ 13	70	4
4	–	♀ + ♂ 10	60	7
5	FSH[c]	♀ + ♂ 10	30	7
6	LH[c]	♀ + ♂ 10	30	7
7	FSH[c] + LH[c]	♀ + ♂ 10	30	7

[a] Gonads of both sexes of 8-, 10- and 13-day-old embryos which were cultured during 4, 7 and 14 days as controls, are not included in this Table. [b] The few explants which did not culture satisfactorily are not included. [c] Purified FSH and LH kindly supplied by N.I.H., Bethesda, U.S.A.

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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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