^{3}H-Thymidine incorporation (DNA synthesis) and radiotoxicity in the ovary of the Japanese quail before and during follicle formation

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Summary. No evidence was found for ribosomal DNA amplification in the oocytes of the Japanese quail, before or during folliculogenesis. DNA synthesis in the somatic cells, involved in follicle formation, starts at the medullar side of the basement membrane. The localized sterilization of the quail ovary after administration of ^{3}H-thymidine (^{3}H-TdR) seems to be due to radiation-induced lesions in the follicle forming somatic cells, rather than to direct radiation damage of the oocyte.

Long before follicles appear in a bird's ovary, the 3 major components that will surround each oocyte individually, are already present: a) the epithelial layer from which follicle cells will develop[2]; b) the ill-defined surrounding ovarian stroma in which thecal cells will differentiate; c) the basement membrane lying between both. In the Japanese quail ovary, the first intrafollicular oocytes are seen just after hatching (i.e. after 17 days incubation). They are localized in the central part (most advanced in development) of the ovarian cortex. Towards the ovarian rim (where the cortex recurves posteriorly), the oocytes are progressively found in a less advanced stage of development. In the days following hatching, the number of follicles increases steadily and an intense cellular activity may be anticipated. This incited us to make an autoradiographic study of the DNA synthesis, after the administration of ^{3}H-TdR, in the ovarian cells playing a role during folliculogenesis.

Material and methods. Through a hole in the shell over the air space, 50 µCi of ^{3}H-TdR-6 (25 Ci/mM) in 50 µl distilled water, were placed on the air space membrane of Japanese quail eggs containing 12-16-day-old embryos. Also 1-4-day-old female Japanese quails received in their neck region, 1 or more s.c. injections of 100-200 µCi ^{3}H-TdR (25 Ci/mM). 1 h to several days after the last application, the female embryos or quails were killed by decapitation. After opening their abdomen, the left ovary was removed and fixed in acetic-alcohol (1:3 v.) for 1 h. After embedding in paraffin, the ovaries were serially sectioned at 6 µm thickness, in a direction perpendicular to their long axis. Part of the sections was stained with PAS or Unna for cytological investigation before autoradiography. The sections were coated with nuclear emulsion L4 (Ilford, England) by the dipping method. After 5-30 days exposure and photographic development, the sections were coloured with methylgreen or iron hematoxylin and eosin.

Results and discussion. 1 h after a ^{3}H-TdR application to a 12-day-old quail embryo, numerous medullar cells are labelled. Most labelled cortical cells are localized at the ovarian rim. The labelling in the latter cells is partly due to premitotic and premeiotic DNA synthesis[3]. Also numerous surface epithelial cells, probably destined to migrate into the cortex, are labelled. 4 days after a similar ^{3}H-TdR application (thus in a 16-day-old embryo), the central part of the cortex ovarii presents numerous labelled necrotic cells and also labelled enlarged meiocytes, not found in control ovaries. These cells resemble the 'blown-up' cells described after irradiation in other tissues[4,5] and are characterized by their enlarged nucleus and cytoplasm. In the medulla, immediately below the central part of the cortex ovarii of 16-day-old untreated embryos, a small number of clearly individualized, basophilic cord-like cell groups appear (figure 1). In these cords, no germ cells can be seen. They often contain dividing somatic cells and are separated from the surrounding medullar cells by an intensely staining PAS positive basement membrane. They seem to belong to one of the types of interstitial mother cells[6,7]. In the ovary of 16-day-old quail embryos, the number of labelled nuclei, 1 h after a ^{3}H-TdR application, is much lower than in 12-day-old embryos or in female baby quails a few days after hatching. Usually some labelled nuclei are found at the rim of the ovary and in the above-mentioned subcortical basophilic cell cords.

During the days following hatching, after an in ovo administration of ^{3}H-TdR to a female 16-day-old quail embryo, the number of labelled cells in the central part of the ovary increases. This is probably due to dividing of already labelled cells, but may also indicate the existence of a ^{3}H-TdR pool during the perinatal period. Autoradiographs of sections of ovaries (fixed 1-2 h after a ^{3}H-TdR injection) from 2-4-day-old quails present 3 zones with a distinct labelling pattern. A 1st zone consists of the central part of

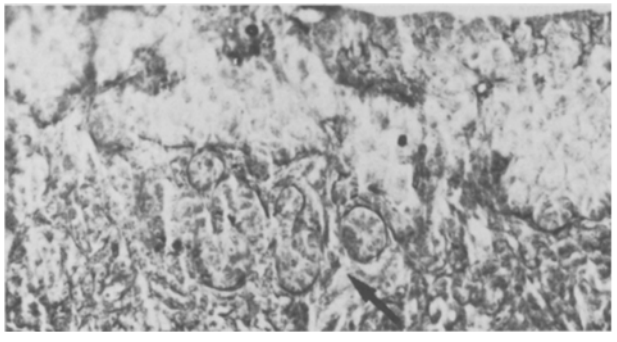

Fig. 1. Section through the central part of the ovary of a 16-day-old quail embryo. Arrow indicates a group of basophilic subcortical cords. PAS-methylgreen, × 210.

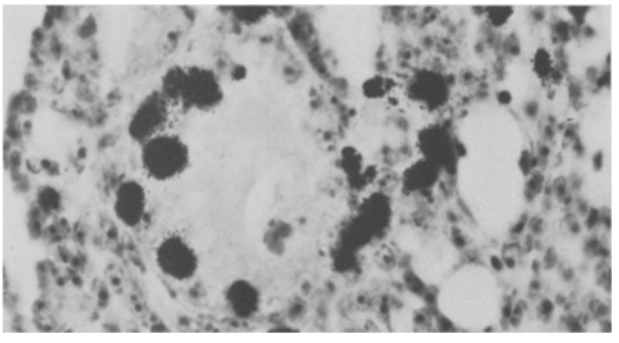

Fig. 2. Autoradiograph of section through the central part of the ovary of a 3-day-old baby quail, 2 h after a s.c. injection of ^{3}H-TdR. Iron hematoxylin and eosin, × 660.

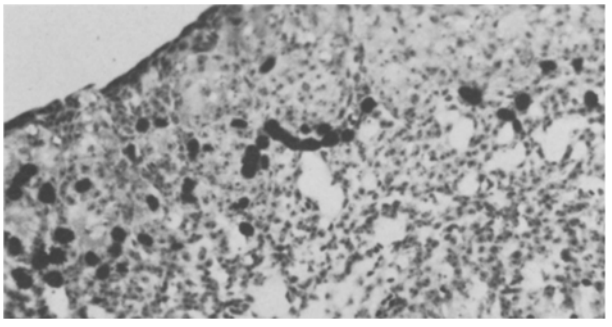

Fig. 3. Autoradiograph from an ovary under the same conditions as in figure 2. Development is gradually more advanced from right to left. Labelling starts in the lacunar zone of the medulla (right), × 210.

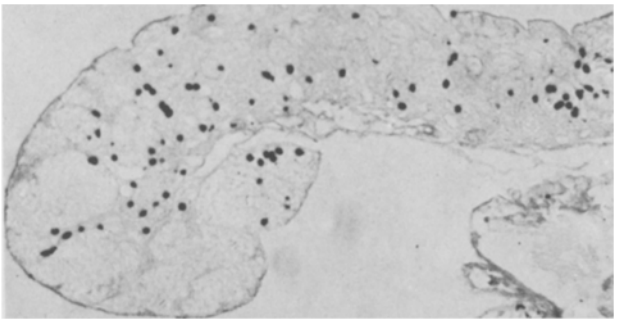

Fig. 4. Autoradiograph from an ovary under the same conditions as in figure 2. Note the 3 zones with a distinct labelling pattern. PAS stain, × 105.

the ovarian cortex and adjacent medulla. Here the 1st follicles are formed deep in the cortex, in the neighbourhood of the basophilic cell cords of the medulla. Enclosed within the basement membrane, numerous labelled follicle cell nuclei are seen (figure 2). Usually several such nuclei are found in each freshly formed follicle (synchronization). At the outside of the basement membrane, also numerous labelled nuclei are present. It is known that ovarian interstitial cells may arise from wall epithelial cells of the medullary cord lacunae[7,8]. In our experiments, some of the latter cells are labelled and in more developed parts of the ovary successively encircle part of the cortex or partially surround early follicles (figure 3). The 2nd zone consists of the dorsally recurved borders of the ovarian cortex. In this region, labelling can be found in the same cell types as has been described in the ovarian rim of the embryos. A 3rd zone, approximately corresponding with the ovarian rim, is a transitional zone localized between both other zones. Here the cortex, only interrupted by very little intervening stromal tissue, is massive and contains meiocytes in the relatively long-lasting pachytene stage. Labelling is usually only seen in the intervening stroma cells and in the adjacent medulla (figure 4). Here also the labelling pattern seems to indicate that the follicle forming process starts in the medulla. Once they have performed their premeiotic DNA synthesis, the oocyte nuclei no longer incorporate ^{3}H-TdR. This indicates, by contrast to what has been found in amphibia[9,10], that no specific gen amplification for ribosomal DNA occurs in quail oocytes during the considered period. In the ooplasm of the early intrafollicular oocytes, however, sometimes a faint labelling over the Balbiani complex (probably indicating mit-DNA synthesis) can be discerned, after ^{3}H-TdR application. We have described a similar labelling pattern in the smallest oocytes (prelampbrush stage) of adult quails[11].

When large quantities of the radioactive precursor are administered, labelled pycnotic nuclei appear about 18 h later, in the region where follicles are developing. This indicates a direct action of the incorporated ^{3}H-TdR on the involved ovarian cells and not necessarily an effect via gonadotropic cells, localized outside the ovary. 2½ days after the injection of the DNA-precursor, the central part of the ovary is almost completely devoid of oocytes and anovular follicle cell cords are seen. During the days that follow a single massive application of ^{3}H-TdR, the zone where radiolesions appear enlarges progressively. This may be due to ^{3}H-TdR recycling: the precursor released by heavily labelled cell nuclei undergoing radionecrosis is reincorporated during the S phase of nuclei in the immediate neighbourhood. Indeed, other instances of ^{3}H-TdR transfer between cells have been shown to occur[12]. However, also the possibility of an extra- or intra-ovarian pool formation must be considered. The sterilization of the central part of the postnatal ovary, after administration of ^{3}H-TdR, seems not to be the result of a direct action on the intrafollicular oocytes, since their nuclei are not labelled. By contrast we think that, first of all, radiolesions or radionecrosis occur in the intensely labelled nuclei of some of the surrounding freshly developed follicle cells and/or theca cells. This probably results in severe biochemical and/or mechanical imbalances with the enveloped oocytes. Death of the latter usually follows rapidly and the remainder of the follicle cells form an anovular follicle. The final effects on the morphology of the ovary are similar to what has been described after X-irradiation in the chicken[13]. Our results also correspond with the observations after X-irradiation in mammals where early primordial follicles represent the most radiosensitive phase[14–16]. Synchronization in follicle formation makes the ovary more susceptible to radiation damage[17,18]. The appearance of anovular follicles could suggest that the oocytes are very sensitive to irradiation. According to Baker's[18] hypothesis, the radiosensitivity of the oocytes should depend on the morphology and metabolic activity of their chromosomes. However, our study indicates that, also during X-irradiation, the direct influence on the early follicle forming somatic cells must be taken into consideration and may even be more important than the direct effect on the oocyte.

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