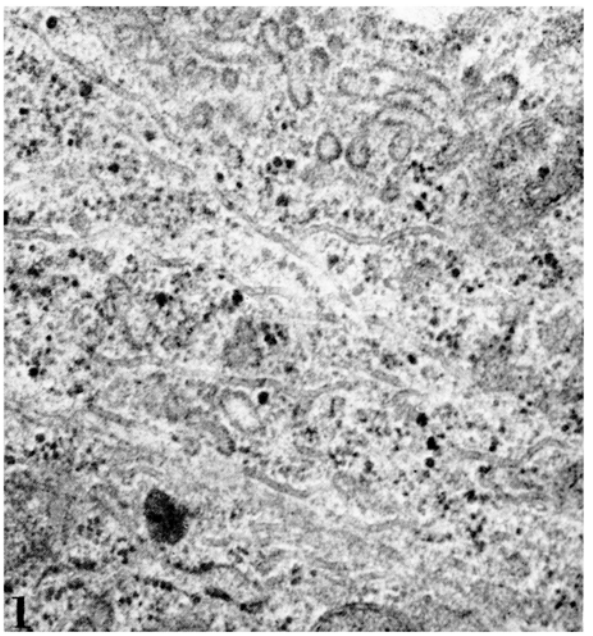

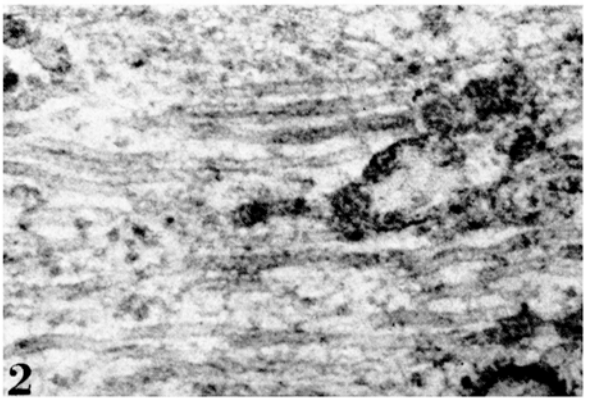

Fig. 1. Microtubules in a neural plate cell of a treated embryo. They follow a sinuous and rather tortuous path. ×41,000.
Fig. 2. Microtubules in a neural plate cell of a control embryo. ×77,000.
Fig. 3. Tortuous microtubules of treated embryos exhibiting notched walls (arrows). ×77,000.

seen but highly sinuous, tortuous and rather crooked microtubules were frequently observed (Figure 1). In such cases, especially when the tubules exhibited a sharp change in direction, the microtubular wall showed small characteristic notches (Figure 3). Their number did not seem to vary and other attributes, such as their width and density to the electrons, were not modified. It could not be ascertained, however, whether these peculiarities affected the shape of the cells in any way.

Comments. The only ultrastructural alteration observed in the neural plate cells of dithiodiglycol-inhibited chick embryos was localized in microtubules. The usual slightly wavy path and smooth tubular wall became tortuous and irregular. It is possible that the oxidizing effect of dithiodiglycol could affect the thiol groups reportedly involved in the polymerization of the tubular element[12].

It could not be ascertained from our experiments whether the observed microtubular modifications had, in any way, a bearing on the inhibition of the morphogenetic movements. Complex biochemical events, such as interference with –SH containing enzymes for instance, could have occurred and not be detected by our technical approach[13].

Résumé. La neurulation a été inhibée chez des embryons de poulet par des traitements au dithiodiglycol ($10^{-3}M$). L'analyse ultrastructurale révèle que seuls les microtubules subissent des modifications.

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Autoradiographic Evidence for Cytoplasmic DNA Synthesis During the Early Final Growth Period in the Oocytes of the Japanese Quail (*Coturnix coturnix japonica*)

In previous studies[1,2] we have established the existence of peculiar subcortical cytoplasmic organelles, appearing in the oocytes of regularly laying Japanese quails just before and/or at the beginning of yellow yolk formation. By an autoradiographic study[1] these RNA-rich organelles[2] have been found labelled 90 min after an i.p. injection of ^{3}H-thymidine. In the present experiment, laying Japanese quails received an i.p. injection of 1 mCi of thymidine 6-^{3}H (14 Ci/mM, 1 μCi/μl) followed 1 h later by a second injection of 1 mCi of the same precursor. 1 h after the second injection, the birds were killed by decapitation and the germinal discs of oocytes, ranging from 4 to 6 mm diameter, fixed in acetic acid-alcohol (1:3 v) for 3 h. After dehydration, the germinal discs were embedded in paraffin and cut at 7 μm thickness in a direction perpendicular to the plane of the germinal disc. Alternating sections were placed on different slides. These paraffin sections were screened under the dark ground microscope in order to select those sections containing some part of the germinal vesicle. Only these sections, which also contain part of the subcortical cytoplasmic organelles, are employed in this autoradiographic study. After deparaffination, the slides supporting the alternating sections are distributed into 4 groups: 1. Slides without any pre-autoradiographic treatment. 2. Slides immersed in 3% perchloric acid at

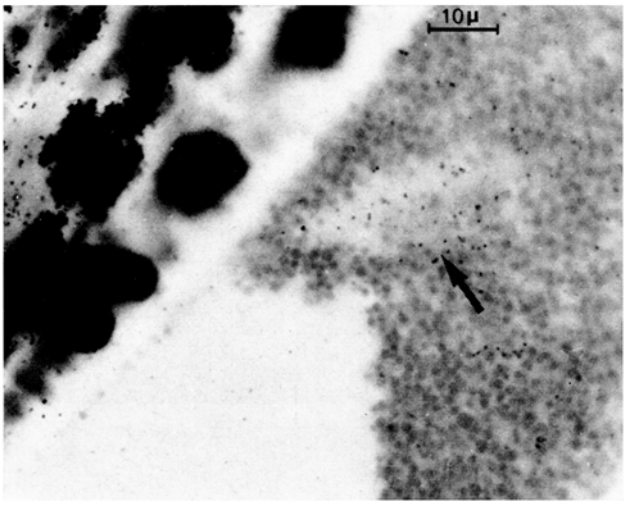

Fig. 1. A section of 5 mm oocyte from a Japanese quail, 2 h after the first i.p. injection of ^{3}H-thymidine, thereafter treated with RNAase and subjected to autoradiography. Part of the germinal vesicle is seen in the lower left corner. Note moderate labelling of the subcortical cytoplasmic organelle (indicated by arrow) and intense labelling of the nuclei in the follicle and theca interna cells.

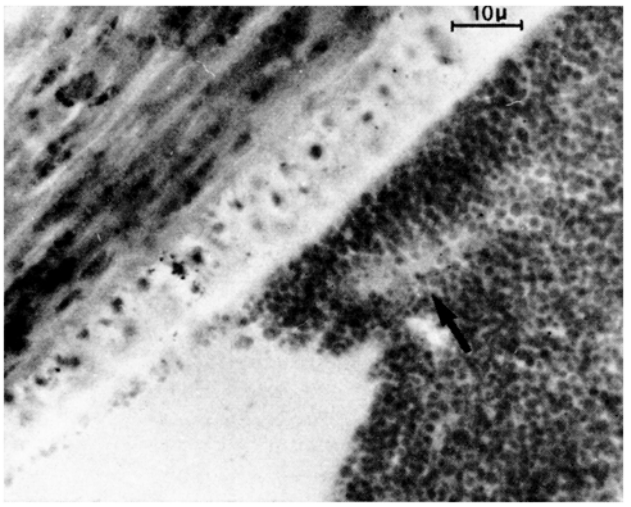

Fig. 2. Autoradiography as in Figure 1, but after treatment with DNAase. Note absence of labelling of subcortical cytoplasmic organelle and feeble residual labelling of some follicle cell nuclei.

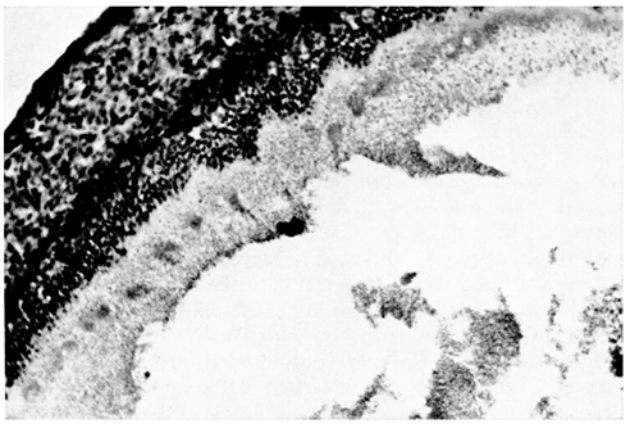

Fig. 3. Section through germinal disc of a quail oocyte with a diameter of approximately 4 mm. Wild objective 10.

4 °C for 20 min in order to extract the acid-soluble precursors 3. Slides immersed in a solution of RNAase (0.2 mg RNAase Sigma per ml of *Tris* buffer at pH 7.5) for 1 h at 37 °C. 4. Slides immersed in a solution of DNAase (0.1 mg DNAase I Sigma per ml of *Tris* buffer at pH 7.5 containing M/300 Mg Cl_2) for 3 h at 37 °C.

The RNAase treatment was intended as a test of purity for the DNAase, i.e. to detect its eventual contamination by traces of RNAase. In addition, it served to rule out the possible incorporation of tritium-labelled impurities into newly synthesized RNA in these RNA-rich organelles. It was essential to check this fact owing to the large quantities of ^{3}H thymidine of high specific radioactivity used. The slides were coated with nuclear emulsion L4 (Ilford, England) by the dipping method. After 30 days exposure and photographic development, the sections were coloured with Groat's iron hematoxylin and eosin.

The subcortical cytoplasmic organelles are clearly labelled (most grains are found at their periphery) on the sections of groups 1 and 2 and this labelling does not disappear after RNAase digestion (group 3).

On the contrary, after DNAase treatment (group 4) the labelling over these organelles disappeared. These facts seem to indicate that the RNA rich subcortical cytoplasmic organelles, although Feulgen negative[2], contain freshly labelled DNA molecules. These DNA molecules are fairly resistant to acid (acetic acid-alcohol fixative, and perchloric acid treatment) and indeed to the whole histological procedure. Since the labelling after ^{3}H-thymidine application appears rapidly (1–2 h pulses) and the other structures of the germinal disc show no appreciable labelling, the moderate, but active DNA synthesis seems to occur directly at the level of these subcortical cytoplasmic organelles. On sections of oocytes of 4 mm diameter, not treated by DNAase, some of the subcortical cytoplasmic organelles at the rim of the germinal disc are found to be almost continuous with the surrounding compact cortical layer (Figure 3). Both the subcortical cytoplasmic organelles and the compact cortical layer in the neighbourhood of the germinal disc present identical staining affinities and a similar pattern of labelling. Thus, although further investigations are necessary to determine the origin of these organelles, these observations provide evidence for at least a partly cortical derivation. Whether the DNA synthesis found in the present experiment is homologous with the unique form of cytoplasmic DNA described by BOND et al.[3] in mouse liver homogenates, or with the informational DNA demonstrated by BELL[4] in embryonic muscle tissue, is not yet clear.

Résumé. Après injection intrapéritonéale de thymidine ^{3}H à la caille japonaise pondeuse, on a trouvé l'existence d'un DNA fraîchement marqué au niveau des organites cytoplasmiques subcorticaux des oocytes au début de leur période de croissance finale.

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[5] The author is very grateful to Prof. L. VAKAET, Department of Anatomy and Embryology, Rijksuniversiteit Centrum, Antwerpen, for his valuable suggestions, and to Mr. G. VAN DEN BROECK, for his technical assistance.