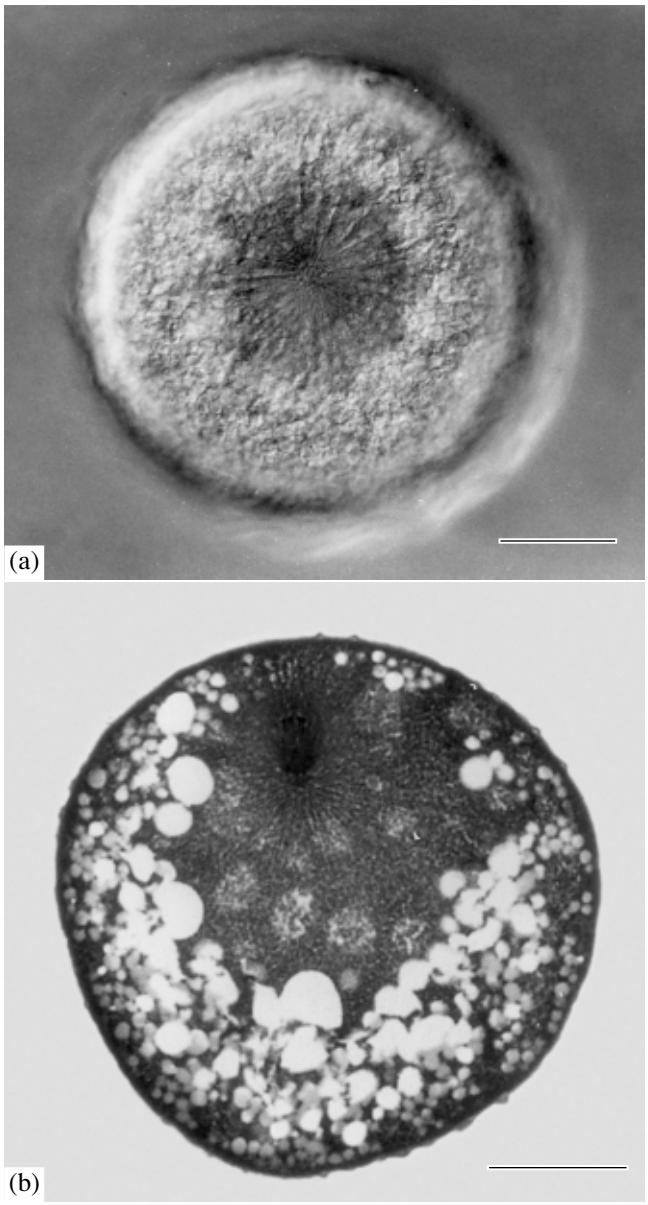


**Fig. 4.** First meiotic division in *N. virens* oocyte: (a) view from the animal pole, DIV; (b) section in the meridianal plane, semithin section, staining by gallocyanin with post-staining by methylene blue.

site where the polar bodies are extruded. As a result of these movements, the peripheral yolk-rich layer and a deeper layer with lipid inclusions are displaced to the equator and appear as a peculiar "pocket" opening in the area of the future animal pole (Fig. 1, *3*).

The second meiotic spindle is formed practically just after the first polar body extrusion and it is located where the first spindle was earlier observed (Fig. 5). Simultaneously, the animal peripheral zone of yolk and lipid-free cytoplasm markedly widens. The second meiotic spindle is shorter than the first and the second polar body, forming within 4 h after insemination, is smaller

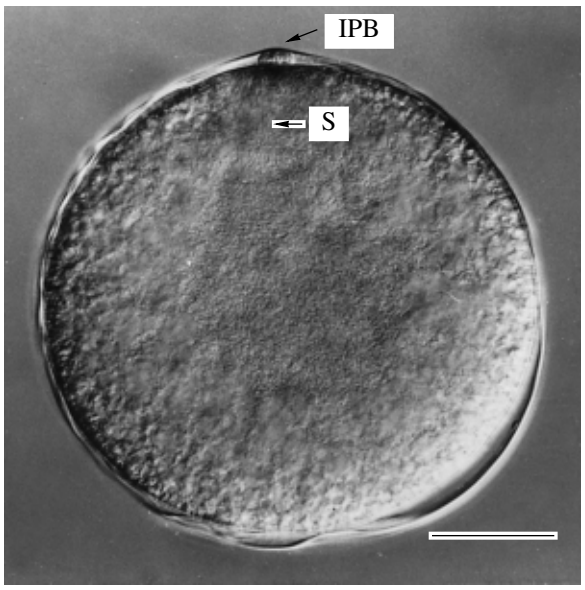


**Fig. 5.** Second meiotic division in *N. virens* oocyte. Designations: IPB, first polar body; S, second meiotic spindle.

than the first. The male pronucleus and spermaster are seen in the cytoplasm of the oocyte (Fig. 1, *4*).

Ooplasmic segregation is completed after the second polar body extrusion. The zone occupied by clear cytoplasm widens and, finally, is fully concentrated in the animal hemisphere. The cytoplasm with lipid and yolk inclusions is displaced to the vegetal pole. At the same time, a relatively large female pronucleus is formed in the center of the animal hemisphere. The enlarged male pronucleus is displaced to the same area. The process of approach and fusion of pronuclei lasts about 20 min and is terminated by the formation of the zygotic nucleus within about 4.5 h after fertilization (Fig. 1, *5* and 6a).

Within 5 h after fertilization, the previous concentric structure of the oocyte, where different kinds of cytoplasm were located as spheres as if enclosed in each other, is replaced by a new stratified structure and the egg acquires a distinct animal-vegetal axis. In the animal hemisphere of the mature egg, which has completed the meiotic divisions, the zygotic nucleus is surrounded by the cytoplasm rich in mitochondria and ribosomes. The cytoplasm with lipid and yolk inclusions is concentrated in the equatorial and subequatorial areas. The vegetal hemisphere periphery is formed by a cytoplasm layer containing relatively small yolk granules. Simultaneously with the concentration of inclusions in the vegetal area, the partial fusion of lipid drops takes place and, as a result, their size increases.

The zygotic nucleus (Figs. 6b and 1, *6*) exists for no more than 15 min and by about 5 h after fertilization, the nuclear envelope has disintegrated. The first cleavage spindle formed in the animal hemisphere has two asters almost equal in size (Figs. 1, *7*, and 7). However,

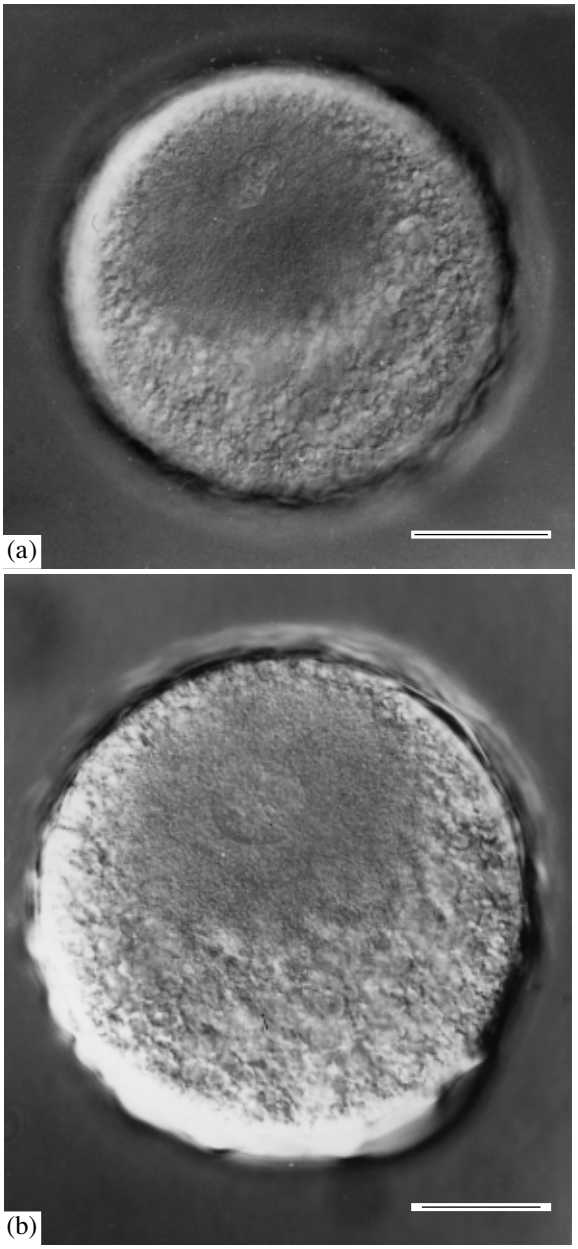


**Fig. 6.** Male and female pronuclei directly before fusion, 4h after fertilization, DIC (a) and *N. virens* zygote, 4.5 h after fertilization (b).

one aster becomes larger by 5.5 h of development. At the animal pole, the zone of clear cytoplasm becomes the widest possible: here, the first cleavage division furrow is laid down by 6 h and the division is completed within 30 min. As a result of the unequal meridional division of the zygote, two blastomeres, which differ markedly in size, are formed: *AB* and *CD*. The larger blastomere, *CD*, inherits the major part of the clear cytoplasm, free of yolk and lipid inclusions. At this stage, the second, dorsoventral, axis of the embryo is

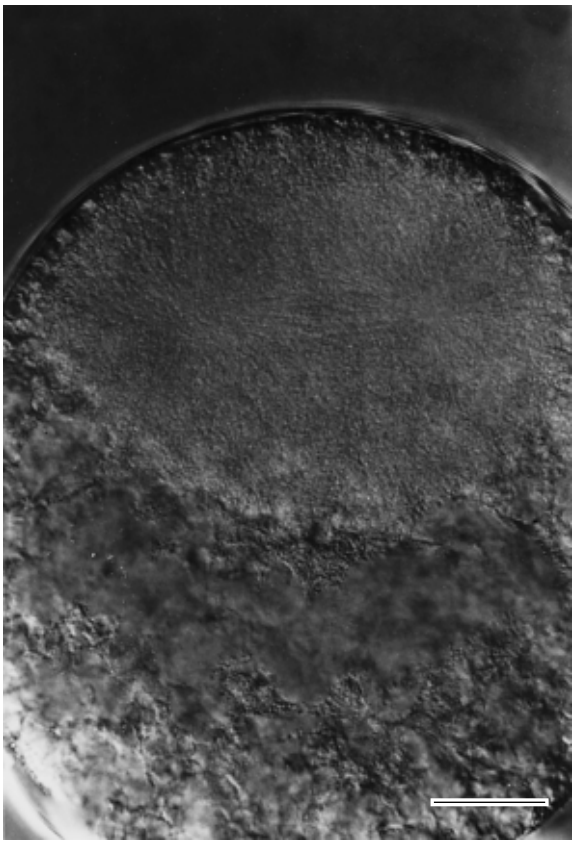

**Fig. 7.** First cleavage spindle in *N. virens*, DIC. Scale: 25 μm.

laid down, which is located perpendicularly to the animal-vegetal axis and to the first cleavage division plane.

**Effects of cytoskeletal inhibitors on ooplasmic segregation.** Colchicine (25 μg/ml) or nocodazole (5 μg/ml) applied within 25 min after fertilization did not stop the cortical reaction, but blocked the movement of inclusion-free cytoplasm for a long time, which started under these conditions only within 3.5–4 h. In the experiments where the oocytes were placed in solutions of these inhibitors after the cortical reaction completion, the appearance of clear cytoplasm on the egg surface was also recorded in some embryos only and only within 4 h after insemination. A certain nuclear material represented by globules of homogeneous substance surrounding the dark stained chromosomes was preserved in the central zone of the oocyte for a long time (Figs. 1a and 8). Later, the number of embryos increased in which the clear cytoplasm reached the periphery and, within 5–6 h of constant soaking in the inhibitor solution (6–7 h of development), the cytoplasm was stratified and the clear cytoplasm was concentrated in one hemisphere of the oocyte.

Unlike colchicine, cytochalasin B blocked the cortical reaction. Within 1 h of soaking in a solution of this inhibitor (0.2 μg/ml), the center of the oocyte was occupied by mixoplasm arising after the nuclear envelope