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Incubator Shelf »Images« in Monolayer Culture

Villy Nielsen and Peter Esser, M.Sc., Senior Scientists, Thermo Fisher Scientific

Under certain conditions of incubation an image of the incubator shelf holes may be formed by cells in monolayer culture. In some cases one observes a lower cell density over the holes (a »positive« image) as shown in Figs. 1, 2 and 4. In other cases one may observe a higher cell density over the holes (a »negative« image) as shown in Fig. 3.

We have investigated these phenomena with L-929 cells and observed that they occur when the culture medium initially has a lower temperature (e.g. room temperature) than the incubator. Differences in heat transfer from the incubator shelf to the culture vessel seem to be the cause, as these special density patterns are seen only in culture vessels placed directly on a metal shelf with holes. We have never seen the patterns in vessels placed on a solid shelf, or in vessels placed on top of other vessels.

We have also observed that L-929 cells create a positive image when the shelf material is 2 mm aluminium (Fig. 1), and a negative image when the material is 1 mm stainless steel (Fig. 3). It would therefore appear that some characteristics of the shelf material, such as heat conductivity, specific heat, and thickness, play a crucial role in producing these patterns, but as to the exact mechanism, there is still uncertainty.

We have observed that the patterns are already established after a few hours incubation, which indicates that the phenomena take place during sedimentation

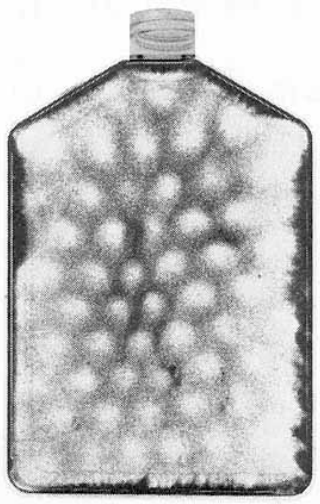

Fig. 1
L-929 cells in 175 cm² flask on aluminium shelf.

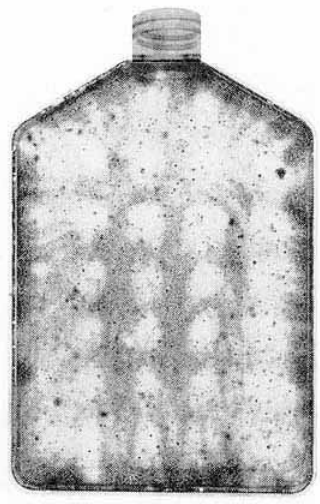

Fig. 2
Primary chicken embryo cells in 175 cm² flask on aluminium shelf.

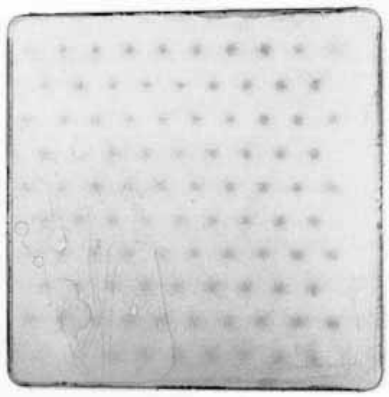

Fig. 3
L-929 cells in 500 cm² square dish on stainless steel shelf.

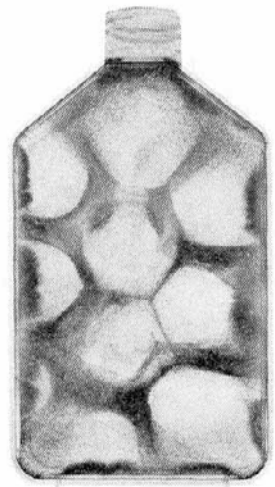

Fig. 4
Unknown cell line in 80 cm² flask on shelf with hexagonal holes. Shelf material unknown.

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and attachment of the cells. It is unlikely that the density variations should be due to differences in growth rate as possible differences in temperature are equilibrated within the first hour of incubation.

One explanation is that the cells during attachment perceive differences in temperature or heat radiation and respond by attaching more densely to the most »pleasant« areas. However, this assumes cellular motility which has not been confirmed.

Another explanation is that convection currents occur in the medium due to uneven heat transfer from the shelf. However, this seems inconsistent with the pattern shown in Fig. 4. This flask was incubated on a shelf with regular hexagonal holes. But can you imagine hexagonal convection patterns?

Whatever the correct explanation may be, the problem can be eliminated by merely placing the culture vessel on a tray in the incubator.

Austria
+43 1 801 40 0

Belgium
+32 53 73 42 41

China
+86 21 68654588

Denmark
+45 4631 2000

France
+33 2 2803 2180

Germany
+49 6184 90 6940

India
+91 22 6716 2200

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Japan
+81 3 3816 3355

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Russia/CIS
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