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Metachromasia in the Living Cell.* (21015)

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It has been generally agreed that metachromatic granules are not observed in the cytoplasm of fixed fibroblasts(1). They, as well as other cells, however, may show diffuse cytoplasmic metachromasia, which has been shown to be due to RNA(2-4). This might indicate that the metachromatic material of the ground substance(5,6) (not due to RNA), which is presumed to be produced by the fibroblast, acquires the quality of a chromotrope only when outside the cell(1). On the other hand, fixed mast cells show intracellular granular metachromasia. They have been presumed to produce both heparin(7,8) and hyaluronic acid(9-11). Mast cells in tissue culture have been identified by the presence of supravital metachromatic granules(12-17). The purpose of this communication is to describe the presence of metachromatic granules in living cells other than mast cells. It is further shown that their presence does not constitute definitive evidence for the identification of cells as mast cells on this basis alone.

Methods. Toxicity of toluidine blue (ToB), and thionin in low concentrations to culture cells has been found to be small. When ToB was added in a final concentration of 1/200000 to a medium of plasma and 20% embryo extract in chick amniotic fluid at the time of explantation, growth observed for 4 days was not inferior to growth in control cultures. Easily observable intracellular metachromasia, however, was not produced until dye concentrations of 1/50000 to 1/100000 were reached, when some toxicity to cells resulted. These concentrations, however, offer the best opportunity for observation of metachromasia and were employed in these studies. Fibroblasts from human skin and from chick skin and heart were cultured in a liquid medium of 20% embryo extract in chick amniotic fluid (18), using the coverslip method. At the second to fourth day of cultivation, the medium was changed to one of 1/50000 ToB in Ringer's solution. A dye concentration of 1/40000 ToB was used when cultures were grown in a plasma medium. Under these conditions, on direct microscopic observation with high power magnification, vital staining started at one side of the fibroblast near the nucleus. First a faintly rose-stained spot appeared in which tiny pink granules were embedded. The granules soon became more distinct and gradually, close to them, violet- or purple-colored granules appeared; the latter

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subsequently came to occupy larger areas on both sides of the nucleus, sometimes filling the whole cytoplasm. The number of rose-stained granules then decreased and often disappeared altogether. The purple of the granules gradually became more intense but many of them could still be seen embedded in a rose-stained matrix. The healthier the appearance of the cells, the longer the rose-colored granules persisted. When nontoxic low concentrations of ToB were used, *i.e.*, below 1/100000, the dye was excreted and the cells continued to grow. When higher concentrations were used, after hours or days, a light blue color first of the nucleoli, then of the nucleus and cytoplasm indicated the approaching cell death. Diffuse orthochromatic staining is evidence of cell death, whereas metachromatic granular staining of the fibroblast is one more criterion of cell viability. Epithelial cells from stomach, intestine, and kidney also showed supravital granular metachromasia, which thus appears to be a general phenomenon in living cells and not connected solely with mesenchymal cells.

Results. Metachromasia in the living cell was influenced neither by pretreatment of the fibroblasts with hyaluronidase† (75 TRU/ml), nor by adding hyaluronidase (75 TRU/ml) to the culture medium. There is no certainty, however, that the enzyme reached the cell interior. Cytoplasmic vacuoles experimentally produced by slight hypotonicity of the medium stained metachromatically with ToB.

When the washed culture of fibroblasts was placed in 0.04 molar NaCl solution, no granular staining with neutral red was seen (19). Similarly in such a medium containing ToB, no metachromasia occurred but, like vital staining with neutral red, it could be produced by increasing the salt concentration of the medium after 10 to 30 minutes of exposure to the hypotonic medium. This would indicate that granular vital staining as well as vital granular metachromasia depends upon an active process in the cell, which could concentrate the dye only from a solution containing a certain concentration of neutral salts. It seems worth mentioning that both metachromatic vital granules and secretory granules first appear close to the nuclear membrane and subsequently diffuse into peripheral parts of the cytoplasm.

When high concentrations of ToB, incompatible with cell life (about 1/5000), were added to living cells in tissue culture, diffuse rose staining of dead cells ensued. Similarly, when orthochromatically stained nonliving cells in tissue culture were subsequently treated with a high concentration of ToB, the blue color of the cells promptly changed to bright rose. Thus, in the dead cell, metachromasia was produced by increasing the dye concentration. In the living cell, variations in dye concentration, in the limits compatible with cell life, change only the speed of the appearance of vital metachromasia. While in nonliving cells changes in pH of highly concentrated ToB solutions lead to loss of metachromasia on the acid side, and increased metachromasia on the alkaline side, pH ranges in the culture medium compatible with cell life have, as a rule, no direct influence on vital metachromasia.

Discussion. It is possible that the action of metachromatic dyes on nonliving cells in tissue culture is, to a certain extent, comparable with their action in model experiments *in vitro,* whereas, with vital metachromasia, factors present only in the living cell are involved. Therefore, once produced, vital granules (*e.g.,* the "crinome") do not subsequently stain metachromatically in dead cells because granular vital metachromasia is dependent upon a continuous process in the living cell.

If we assume that in the living cell dye accumulation may be achieved by an active process in the cell(19-21,26) the present data would be consistent with the view that intracellular segregation of dye and its concentration(22-25) may be a factor sufficient to produce metachromasia. The regular shift, however, from pink towards saturated purple (a saturated purple is produced by a mixture of colors from both ends of the visible spectrum) in the course of vital staining with ToB and the shift from purple to blue at cell death would seem to involve, besides increased density of the granules(23,26-28), possibly

† Hyaluronidase—Wyeth.

also more complicated processes, which at the present time are unexplained. Further work on the mechanism of this process is contemplated.

It appears that, in tissue culture, mast cells (12-15) cannot be specifically identified by supravital metachromasia since the living cells we have examined in tissue culture have all stained metachromatically.

Summary. 1. Intracellular granular metachromasia, appearing in a sequence of two colors, *i.e.*, pink and purple, is a general phenomenon found in a variety of living cells in tissue culture. 2. In tissue culture cells, the appearance of intracellular granular metachromasia is evidence of cell viability and intracellular orthochromasia, one more criterion of cell death. 3. Mast cells, or "precursors" of mast cells cannot be specifically identified by supravital metachromasia. 4. Dye condensation effected by an active process in the cell, as one possible factor in producing vital metachromasia, is discussed.

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