The staining of the living cells depends on many physical conditions. Such a small variation between 0.6 per cent. and 0.65 per cent. of NaCl in Ringer's solution makes a great difference in the vital staining process. The temperature is also important for it seems that each color, to give the best results, must be used at a particular temperature, for instance Cresyl Violet gives best results at the low temperature of 20°C., Diazin Green and Janus Green at 26°C., Natural Red at 32° to 35°C.

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Some changes in the dying cell.

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By ordinary transmitted light the nucleus of the living cell is an optically homogenous body lying in a cytoplasm which is more or less granular. The optical difference between the nucleus and the cytoplasm is more strikingly shown by dark field illumination where the nucleus appear optically empty, whereas the cytoplasm scintillates with bright spots. The cytoplasm has, therefore, been considered to be distinctly heterogeneous in contrast to the optically homogenous nucleus. That this is not true may be seen in the following experiment:

By means of the centrifuge the cytoplasmic granules of the sea urchin egg can be driven to one side of the egg. On cutting away this part one may obtain an egg fragment consisting of protoplasm which is transparent and optically empty, even when viewed with dark field illumination. This fragment is fully capable of developing. We must, therefore, conclude that the cytoplasm may be as optically structureless as the nucleus. When the cell dies, however, a difference in structure with the dark field illumination becomes at once apparent. Coagulating agents, which are not violent in their reaction, such as gentle heat or weak formalin, make the cytoplasm diffusely milky in appearance, owing to the formation of closely packed and uniformly sized spherules. In the sea urchin egg these globules

are about 1 micron in diameter. In tissue cells they are somewhat smaller. The coagulating nucleus in the dying cell, on the other hand, gives a different picture. Here also uniformly sized granules make their appearance but, instead of being evenly distributed, they collect into granular strands which run together to form a nuclear network.

Another striking death change is a change in the nature of the surface layer of the cell. Neutral Red, injected into a living cell, *c. g.*, amoeba, a ciliated cell or a starfish egg, colors the cell a rose red, indicating a P_H on the acid side. On the death of the cell the color changes to orange. In the relatively large amoeba the Neutral Red may even be precipitated out as yellow crystals. This color change may be explained as follows: Death destroys the impermeable surface layer of the cell, upon which the alkaline environing medium diffuses into the freely permeable coagulated mass and reverses the color of the Neutral Red.

Another death change which seems to be peculiarly significant regarding the reducing ability of the different components of the cell is shown in the following experiment: When living cells are placed in a solution of Janus Green the mitochondria in the cytoplasm stain a beautiful blue while the nucleus remains colorless. As soon, however, as the cell becomes moribund the dye penetrates the nucleus but instead of staining it blue it gives to the nuclear network a lilac and sometimes a distinctly reddish hue. Janus Green is an oxidation product of diethyl safranin and can be reduced to the red diethyl safranin. In the healthy cell the dye cannot enter the nucleus. When the cell becomes moribund the resistance of the nucleus to the penetration of the dye is diminished and the reducing action of the nuclear substance then becomes evident. When the cell is quite dead this reducing ability is lost and, as more of the dye penetrates, the red color soon becomes masked so that the nucleus finally colors a deep blue.

The reducing ability of the cell nucleus is also borne out by the reaction of the cell to vital methylene blue. In sublethal doses the readily reducible methylene blue enters the cytoplasm, where it is reduced to its colorless leuco compound. In lethal doses it accumulates in the nucleus so that when the dead cells are exposed to air the nucleus stains more heavily blue than the cytoplasm.