

harmonizing with the CO_2 combining power in both instances. In the first of these cases there was a rise to P_H 7.33 four hours after administration of insulin. In case 6 suffering from uremia following a bladder operation, and dying several hours after the test, the P_H was 7.15 and the CO_2 20. In case 7 suffering from advanced nephritis with marked nitrogen retention (blood creatinine of 9 mg.), the P_H was 7.30 and the CO_2 content 47. In the case with cardiac decompensation there appears to be a slight reduction in the P_H . Case 9, following a cholecystectomy operation and alkali therapy, showed a CO_2 combining power of 98. Six hours later when a P_H of 7.53 was observed the CO_2 had fallen to 87. On the next morning the P_H was 7.46 and the CO_2 was 79.

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Some studies on the vital staining of blood cells.

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There are many papers on the vital staining of the blood cells but, considering the many papers of the conventional method used in the examination of the blood, one may say that the field of the vital staining is rather unexplored. It is surprising to find how few have actually studied living cells. Most of the work on vital staining is in reality *supra vital*, that is to say the living cells on taking up the stain died. I will mention of many investigators the names of Rosin and Biebergeil, Sabin and E. Cowdry. These writers observed cytoplasmic granules which took up certain dyes, while the cells containing them continued to live.

I have made a few comparative studies of the effect of certain vital dyes on the cytoplasmic granules in the white blood cell of man. To introduce the dye into a drop of blood diluted with Ringer's solution, I used the following method, which was

suggested to me by Mr. Chambers: Coverslips were flooded with an aqueous solution of the dye, which was allowed to evaporate, leaving the dry dye evenly distributed on the coverslip. The drop of blood was placed on this coverslip and studied as a hanging drop, suspended in culture slides. The observations were made in a warm chamber.

Out of a dozen various dyes I have found the two following to be the best. They are both American made dyes. Janus Green from the Providence Chemical Laboratories and Cresylecht Violet from the National Aniline and Chemical Co. This American Janus Green is taken up very rapidly. During the first few seconds it colors the cytoplasm diffusely and gradually concentrates on the granules, which finally take up all of the stain, leaving the cytoplasm almost transparent. The eosinophil granules are very prominent and large and take a deep blue color. During this time the cell exhibits amoeboid movements. The granules move but seldom extend into the slender pseudopodia.

After one hour, as the cell begins to die, the Janus Green tends to fade out of the granules and enters the hitherto colorless nucleus, coloring it violet. This is significant because it indicates a reduction of the dye. The neutrophil leucocytes have very fine granules which stain more faintly. These granules exhibit greater activity of movement than those of the eosinophiles. In dead cells, both eosinophil and neutrophil, the nucleus loses its color while the granules again take up the stain and persist as deeply stained granules for a long time.

Cresylecht Violet is more toxic than Janus Green. Its preliminary effect, however, is to stimulate not only the cells but also the cytoplasmic granules to considerable activity.

In the neutrophil cells I have found two distinct kinds of granules, first, one which is apparently identical with that of the eosinophil and second, one which is very small. The large granules may be few or many. Although these granules resemble the eosinophil granules, we cannot consider them as being the same, because, during the decoloration of the cell which accompanies its death these large granules fade out at the same time and in the same way, as the fine neutrophil granules and, therefore, earlier than the granules of the eosinophil cell.

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