ionic membranes from various animals at different stages of gestation be made to complete a survey of the distribution and range of this material. Such studies should provide sound evidence for interpretation of the relation between the endocrine functions of ovaries and placenta in the higher mammals. The publication of quantitative data will be deferred until the completion of additional tests.

This is a preliminary report.

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## Degeneration in Vitro of Leucocytes and Connective Tissue Cells Under the Influence of Light.*

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In an attempt to keep tissue cultures of certain blood cells under constant observation, work was completely blocked by the exceedingly rapid and extreme degeneration of the cells. In this rapid degeneration all of the various types of leucocytes showed degenerative changes rather similar in character, which differed, however, in certain particulars, for each individual type of cell. Of the changes shown by the various types of leucocytes, those shown by the polymorphonuclear neutrophil were the most striking. The neutrophils rapidly became very amoeboid, and at the same time their cytoplasm became more fluid, as was shown by the exaggerated brownian movement of the cytoplasmic granules. Soon cellular movement ceased; the cells rounded and became spherical. The

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polymorphic lobulated nuclei became edematous, rounded, but after a short while began to shrink and became reduced in size, although they still maintained their spherical or slightly ellipsoidal shape. The cells became swollen and edematous, and increased tremendously in size; in several instances the diameter of the cell reached three times that of the cell when it had first become rounded. During the development of this edema a number of minute oil droplets, giving a positive Sudan III reaction, became visible in the cytoplasm. From this stage on, two terminations to the process have been observed: (1) the cytoplasm gradually coagulated around the nucleus, leaving the cell membrane as a shell which slowly autolyzed; (2) the edema became so great as to rupture the cell membrane. At the moment of rupture the dancing granules and oil droplets of the cytoplasm almost instantly coagulated, evidently from contact with the surrounding medium.

Without here going into details of the degenerative changes of the other types of leucocytes, it may be stated that they too followed a more or less similar, though less spectacular course of hydropsical degeneration.

This rapid degeneration has been noted in cultures of leucocytes from guinea pigs, cats and rabbits. In the preparation of the cultures the blood has been drawn under light ether anesthesia, either by heart puncture or through the abdominal aorta. It has then been centrifuged at low speed at room temperature in order to separate the leucocytes, which appear as the characteristic buffy coat. It is from this buffy coat that the hanging drop cultures have been made. Various culture media have been used including Tyrode solution, autogenous serum, autogenous plasma and autogenous heparin plasma plus chick embryo juice in Tyrode solution. The hydrogen ion concentration of all of these media has been set to approximately pH 7.4. It should be noted that in all of these media some red blood cells were brought over with the explant at the time of inoculation.

After the elimination of various preliminary factors which could have been responsible for such a rapid degeneration of the cultures as that described above, including the vaseline used to seal the cultures, cedar oil used for immersion objectives, the temperature at which the cultures were kept and the handling to which they were subjected, it was determined that in almost every case of such degeneration there was a definite record of exposure of the culture, for at least a minute or so, to some fairly concentrated light source, as for instance, a microscope lamp.

With this as a point of attack, an examination of over 700 cultures of white blood cells has shown that exposure of the cultures to rays from a light source (various types of incandescent electric globes) of such intensity as would be used in ordinary visual microscopic work, with the oil immersion lens, results in the rapid and extreme degeneration of the cells. To show how extreme and rapid this degeneration is, the increased motility of the leucocytes, due to the action of light, has been noted a number of times in cells after less than 10 seconds exposure to a light source such as that noted above. In other cases, an exposure of the leucocytes to such a light source, during a preliminary microscopic examination of 1 or 2 minutes, has resulted in the culture showing few or no living cells when examined a number of hours later. In all of these instances, controls from the same set of slides, kept in the dark, have remained normal.

Further, using an optical system which blocked out practically all of the ultra violet and infra red rays, it was determined that this degeneration under the influence of light was caused by the visual part of the spectrum. Using a constant light source, together with red (No. 29), blue (No. 45) and green (No. 58) Wratten light filters, it was further determined that the leucocytes underwent the same type of degeneration upon exposure to light of each of these three parts of the spectrum.

As stated above, all of these cultures contained at least some erythrocytes. This being true, there was a possibility that the leucocytes showed degenerative changes as a result of toxic substances produced by the action of light on these red blood cells. In order to analyze this possibility, cultures of leucocytes were inoculated with autogenous red blood cells which had been previously exposed to the action of intense light for about 5 hours. These cultures were then kept in the dark until examined. Upon examination at the end of 12 hours, the cultures showed no signs of any degeneration, such as that produced by the action of light upon white blood cells, indicating that the degeneration of the leucocytes as described above was not produced by toxic substances resulting from the action of light on the erythrocytes.

It next seemed desirable to determine whether this degeneration produced by the action of light upon the leucocyte was an intrinsic property of the cell itself, or whether it was the result of sensitization of the cell by some substance, *e. g.*, hemoglobin, furnished by the accompanying red blood cells. Inasmuch as at that time we were unable to obtain leucocytes free from red blood cells, recourse was had to fibroblasts cultured from chick embryos of from 4 to 14 days

incubation. These fibroblasts from the heart, trunk and intestine of the chick embryos, grown in Tyrode solution, were exposed to the same light source which caused death of blood cells in from 5 to 80 minutes. At the end of 18 hours the fibroblasts were practically normal. On the other hand, cultures from the same groups of slides, inoculated in Tyrode solution plus autogenous washed red blood cells, when exposed to the same light source, showed a striking degeneration in from 1 to 3 hours. In this process of degeneration the cell either became packed with water vacuoles, or else the cell contents showed the same increased fluidity as that shown by the degenerating leucocytes. In both cases the cells gradually rounded, and both nucleus and cytoplasm became edematous. As may be seen from these observations, this type of degeneration of the fibroblast appears to be practically identical in nature with that described for the leucocyte under the influence of light.

Furthermore, it was found that these sensitized fibroblasts reacted to the red, the green and the blue components of the visible spectrum, and to the white light itself (the infra red and the ultra violet being blocked out), in a manner wholly comparable to that shown by the leucocytes.

This is a preliminary report.

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## On the Experimental Production of Showers of "Non-Motile" Leucocytes.*

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Schilling,[1] in 1908, while studying the degeneration of white blood cells under the dark field microscope, found that dying leucocytes showed a definite swelling of the nucleus, and a change in the size and refractivity of the specific granules. Similar changes were later described by Sabin[2] as taking place in the death of the polymorphonuclear neutrophilic leucocytes. In the latter case, these changes were observed while studying the white blood cells by means of the supra-vital technique. Sabin was able to follow every step in the change from the living, motile polymorphonuclear neutrophilic

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