formulated in terms of a continuously changing sol-gel system (Mast)[10] and this concept has been further developed and applied by Lewis[11] to rat fibroblasts. Since gels probably acquire their rigidity from a structure of interwoven fibers, this does not basically differ from the theory of the fibrillar structure of cytoplasm.

[10] Mast, S. O., *Protoplasm*, 1931, **14**, 321.

[11] Lewis, W. H., In the Structure of Protoplasm. A Monograph of the Am. Soc. of Plan Phys., Iowa State College Press, 1942.

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The Structure of Unstained Reticulocytes.

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Brilliant cresyl blue and certain other basic dyes, if applied to fluid blood, bring out a reticulum in young red blood cells. This reticulum fails to appear if brilliant cresyl blue or other basic dyes are applied to dried and alcohol fixed blood smears. Moreover, no reticular structure could hitherto be demonstrated in the young red blood cells by microscopic examination of unstained blood with direct or oblique illumination, dark field illumination or in ultraviolet light.[1]

While examining unstained wet blood films of albino mice with the phase microscope, we found that an occasional red blood cell showed a very delicate, hardly discernible reticulum. The cells showing this reticulum were fewer in number than the reticulocytes demonstrable by supravital staining with brilliant cresyl blue. However, when certain hypotonic salt solutions, preferably potassium oxalate 0.8% or ammonium oxalate 1.2% were mixed with the fresh blood, a larger number of cells, corresponding to the percentage of reticulocytes, showed distinct granules and short rods within their cytoplasm.

Most of these rods and granules showed prominent Brownian movement. In such preparation, varying number of cells showed marked loss of hemoglobin. Granules predominated in hemolyzed cells, while rods were more prominent in cells containing ample hemoglobin. The percentage of cells showing rods or granules was higher in those areas of the preparation in which hemolysis was marked. Smears, dried and fixed in alcohol, failed to show these cytoplasmic structures. However, dried but unfixed smears, if mounted in 10% formalin or 1.2% ammonium oxalate, (Fig. 1), showed rods and granules resembling those seen in the fresh preparations. Also, fixation of dried smears in 3% aqueous potassium dichromate did not interfere with the demonstration of the rods and granules. It is noteworthy that both formalin and dichromate applied to the dried smear hemolyzed the red blood cells. Formalin added to fresh blood

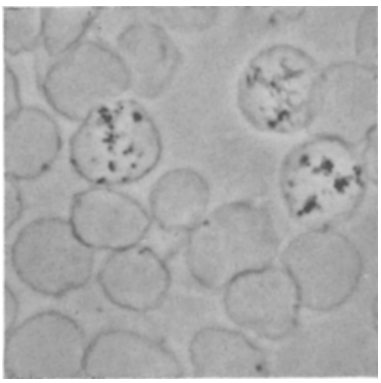

FIG. 1.
Phase illumination of dried film of mouse blood mounted in 1.2% ammonium oxalate.

[1] Isaacs, R., in Downey, Handbook of Hematology (New York: Paul Hoeber), 1938, **1**, 16.

prevented hemolysis, but also prevented the demonstration of rods and granules when blood so treated was examined in either wet or dried films.

The identity of the cells containing rods and granules with reticulocytes could be demonstrated by adding a small amount of brilliant cresyl blue or new methylene blue[2] to the oxalate solution used in preparing wet films. When this was done, the rods and granules were incorporated into stainable reticulum. Even small amounts of these basic dyes stained the reticulocytes very rapidly and only an occasional cell could actually be observed in the process of staining. From such observations it appeared that some of the granules seen in unstained preparation corresponded to the metachromatic granules seen in the midst of the reticulum when stained with basic dyes.[3] The reticulum, however, appeared frequently independently of the preexisting granules, although these granules may be incorporated into the meshes of the reticulum.

Preliminary experiments demonstrated essentially similar rods and granules in human reticulocytes.

[2] Brecher, G., unpublished data.

[3] Cesaris-Demel, A., *Folia hemat.*, 1907, **4**, Suppl. 1, p. 1.

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Influence of Interrupted Carcinogenic Treatment on Tumor Formation.*

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There is considerable evidence that the formation of cancer occurs as a series of biological changes.[1-5] This concept is supported by several types of experimental data but of special consequence is the evidence of a latent period under certain conditions of carcinogenesis. When a carcinogen is applied to the skin of mice for a time short of that necessary to induce tumors, the subsequent resumption of the carcinogenic stimulus will quickly evoke the formation of tumors even though 2 to 3 months intervene between the 2 periods of treatment.[6] Actually a noncarcinogenic agent, such as heat, a wound or croton oil, may substitute for the carcinogen in the second period.[7-8] Such observations indicate that the initial application of the carcinogen produces alterations in the cells that persist for a considerable period. Whether the initial cellular changes revert gradually or persist indefinitely is not known. In order to test this point, rest periods from one to 3 months were interspersed between 2 periods during which the carcinogens were applied to mice at minimal carcinogenic levels. It was thus possible to obtain some information on the reversibility of the process.

Procedure. This investigation may be divided into two parts: in one, methylcholanthrene was employed to initiate the process

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[1] Berenblum, I., and Shubik, P., *Brit. J. Cancer*, 1947, **1**, 383.

[2] Friedewald, W. F., and Rous, P., *J. Exp. Med.*, 1944, **80**, 101.

[3] Rusch, H. P., *Physiol. Rev.*, 1944, **24**, 177.

[4] Kline, B. E., and Rusch, H. P., *Cancer Res.*, 1944, **4**, 762.

[5] Rusch, H. P., and Kline, B. E., *Arch. Path.*, 1946, **42**, 445.

[6] Lavik, P. S., Moore, P. R., Rusch, H. P., and Baumann, C. A., *Cancer Res.*, 1942, **2**, 189.

[7] Des Ligneris, M. J. A., *Am. J. Cancer*, 1940, **40**, 1.

[8] Berenblum, I., *Arch. Path.*, 1944, **38**, 233.