16602 P

Histologic Evidence for the Synthesis of Protein in Lymphocytes Following Parenteral Injection of Antigen.

T. N. HARRIS AND SUSANNA HARRIS.

From The Children's Hospital of Philadelphia (Department of Pediatrics, School of Medicine, University of Pennsylvania), Philadelphia, Pa.

Experimental evidence for the formation of antibodies in the local lymphatic system following the parenteral injection of antigen was presented by McMaster, *et al.*,[1,2] and by Ehrich and Harris.[3] Dougherty, Chase and White[4] demonstrated antibodies in lymphocytes, and Harris, *et al.*,[5] showed that lymphocytes were a primary site of antibodies following the local injection of antigens. Other cells of mesodermal origin have, however, been regarded as the possible sources of antibodies: macrophages[6] and, more recently, plasma cells.[7,8]

The recent applications of histochemistry to physiology have offered an additional approach to this problem. It was first shown by Caspersson[9,10] and Brachet[11] that cells which were actively forming new protein were characterized by large amounts of ribonucleic acid in their cytoplasm. Additional evidence for the association of cytoplasmic ribonucleoprotein with protein synthesis has been reviewed by Greenstein[12] and by Dempsey and Wislocki.[13] Since there is considerable immunologic evidence of the production of antibody by the popliteal lymph node of the rabbit[3,14,15] following the injection of antigens in the foot pad, and since a tissue in which antibody is being formed could be expected to show evidences of protein synthesis, it seemed feasible to attempt to identify the cells involved by histochemical methods.

Accordingly, a study was undertaken of the localization and concentration of ribonucleic acid in the cells of the popliteal lymph node, in correlation with the production of antibody by that node, after the injection of antigens into the local tissue. This preliminary report will present a brief description of the histologic changes in the lymph node during the first few days after the injection of the antigen.

Methods. Injections of influenza virus into the pads of rabbit feet and the collection of regional (popliteal) lymph nodes were made as described elsewhere.[15] Each animal in this series received the antigen only in one foot, so that the contralateral lymph node could serve as a control specimen. Animals were sacrificed at 1, 2, 3 and 4 days after the injection of antigen. Both popliteal lymph nodes were excised. Parallel sections of each node were stained by Azur II-Eosin and methyl-green-pyronine respectively. The latter set of stains is for the respective nucleic acids: methyl green stains desoxyribonucleoprotein a somewhat bluish green, and pyronine stains ribonucleoprotein red.

Results. The histological findings in the Azur-Eosin stained sections of these popliteal

1 McMaster, P. D., and Hudack, S. S., *J. Exp. Med.*, 1935, **61**, 783.

2 McMaster, P. D., and Kidd, J. G., *J. Exp. Med.*, 1936, **66**, 73.

3 Ehrich, W. E., and Harris, T. N., *J. Exp. Med.*, 1942, **76**, 335.

4 Dougherty, T. F., Chase, J. H., and White, A., PROC. SOC. EXP. BIOL. AND MED., 1944, **57**, 295.

5 Harris, T. N., Grimm, E., Mertens, E., and Ehrich, W. E., *J. Exp. Med.*, 1945, **81**, 73.

6 Sabin, F. R., *J. Exp. Med.*, 1939, **70**, 67.

7 Bjoerneboe, M., Gormsen, H., and Lundquist, F., *J. Immunol.*, 1947, **55**, 121.

8 Fagraeus, A., *J. Immunol.*, 1948, **58**, 1.

9 Caspersson, T., *Naturwissenschaften*, 1941, **29**, 33.

10 Caspersson, T., *Chromosoma*, 1941, **1**, 605.

11 Brachet, J., *Arch. Biol. Paris*, 1942, **53**, 207.

12 Greenstein, J. P., *Advances in Protein Chemistry*, 1944, **1**, 210.

13 Dempsey, E. W., and Wislocki, G. B., *Physiol. Rev.*, 1946, **26**, 1.

14 Ehrich, W. E., and Harris, T. N., *J. Exp. Med.*, 1946, **83**, 373.

15 Harris, S., and Harris, T. N., in press.

lymph nodes have been reported elsewhere[15] and will not be repeated here. On examination of the sections of normal nodes by methyl-green and pyronine, almost no pyronine stained material could be found.

The histologic differences among the stimulated nodes were primarily those of degree. In these nodes, one day after the injection of antigen, a few young lymphocytes were seen to contain red granules in the cytoplasm, and in many of these cases, clearly defined red nucleoli. Such cells were progressively more numerous on the second, third and fourth days. On these latter days there was an occasional crescentic accumulation of pyronine-staining material at the very edge of the nucleus of cells which showed this material, and occasionally cells were seen which showed no cytoplasm except for a sector of material solidly stained red, contiguous to about one-quarter of the circumference of an otherwise denuded nucleus.

No plasma cells were seen in the greatly enlarged cortex of the stimulated lymph nodes by either method of staining. The few plasma cells noted were in the medullas of lymph nodes, which were fairly similar in area to those of control nodes, and the number of these cells did not differ remarkably, in these preparations, between control and stimulated nodes.

The great majority of cells with pyronine-staining granules were young lymphocytes. A few more mature, smaller, lymphocytes showed these granules, on the third and fourth days, and a number of the cells with these granules had nuclei so vesicular as to suggest that they were transitional forms between the reticulum cells on one hand and clearly recognizable young lymphocytes on the other. Within this range the distribution was continuous: A complete series of cells with pyronine-staining cytoplasmic granules could be picked out of a given section, from transitional forms to mature lymphocytes. Large lymphocytes of various stages were certainly much more in evidence than the mature cells among those with ribonucleic acid granules.

It is well to recall that the pyronine-staining granules are not the new protein, or antibody, itself, but a part of the mechanism which produces this protein. Accordingly it is quite consistent with our interpretation that the ribonucleic acid granules are found primarily in young rather than old lymphocytes.

Summary. The identification of the cell within lymphatic tissue which produces antibody has been approached by the application of a new histochemical technic for localization of cytoplasmic ribonucleic acid. The latter has been found to be an invariable concomitant to the formation of new protein in tissue. In lymph nodes actively engaged in the production of antibodies a wide range of lymphocytes, largely younger forms, was found to have cytoplasmic granules and nucleoli stained with pyronine, which is used to identify ribonucleic acid. Such granules and nucleoli were found also in transitional forms between reticulum cells and lymphocytes, but in no other cell types.

16603

Viscosity Studies of Erythrocytes from Persons with Sickle Cell Disease.

WILLIAM M. MCCORD, WILLIAM H. KELLEY, PAUL K. SWITZER, AND F. BARTOW CULP. (Introduced by T. G. Bernthal.)

From the Departments of Chemistry and of Medicine, Medical College of the State of South Carolina, and of Roper Hospital, Charleston, S. C.

The viscosity of the erythrocytes of sickle cell disease varies with changes in oxygen tension as Ham and Castle[1,2] have shown. The variation in viscosity is due to changes in

1 Ham, T. H., and Castle, W., *Trans. Assn. A. Phys.*, 1940, **55**, 127.

2 Ham, R. H., and Castle, W., *J. Clin. Invest.*, 1940, **19**, 788.