that albumin stimulates this state and in some unknown way produces the effects described on the host.

Summary. Parenteral administration of heterologous albumin (125 mg/kg body weight per day or 2 g/kg body weight in 14 days) was found to increase the incidence of tumors produced by inoculation of sarcoma 180 cells from 59% in control mice given saline, to 92% in mice given albumin. In the mice given Ehrlich's ascites tumor cells the take was 54% in animals given saline but was increased to 91% in animals receiving albumin. Similar but less significant results were obtained with hydrolyzed albumin.

Prior incubation *in vitro* of albumin with these tumor cells did not produce an enhancement in takes on subsequent inoculation in mice, suggesting that the albumin did not act directly upon the tumor cell. It appears that the effect of the heterologous albumin is more likely to be due to an albumin-host interaction than an albumin-cancer cell effect. This is supported by the study of the effect of albumin on growth of the tumor *in vivo* associated with a decrease in weight of the adrenals and ovaries. This finding is being investigated.

1. Ledoux, L., *Nature,* 1955, v176, 36.
2. ———, *ibid.,* 1955, v175, 258.
3. Cole, W. H., McDonald, G. O., Roberts, S. S., Southwick, H. W., *Dissemination of Cancer, Prevention and Therapy,* Appleton-Century-Crofts, Inc., New York, 1961.
4. Tannenbaum, A., Silverstone, H., *Cancer Res.,* 1949, v9, 162.
5. ———, *Advances in Cancer Res.,* 1953, v1, 451.
6. Ghose, T., Nairn, R. C., Fothergill, J. E., *Nature,* 1962, v196, 1108.
7. Gazet, J-C., M.S. Thesis, Univ. of Illinois, 1963.
8. Buinauskas, P., McDonald, G. O., Cole, W. H., *Ann. Surg.,* 1958, v148, 642.
9. Slawikowski, G. J., *Cancer Res.,* 1960, v20, 316.

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Acid Ribonuclease Activity in Peripheral Leucocytes of Mice: A New Cytochemical Technique.* (29025)

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Using spectrophotometric methods for determination of ribonuclease activity Houck and Berman(1) described the concentration of this enzyme in human leucocytes. This method permits quantitative recovery of the enzyme in the cell. Later, Novikoff(2) described the presence of this enzyme in specific granules of the ploymorphonuclear leucocytes in peritoneal exudates of rabbits. Cohn and Hirsch(3) described the lysosomal nature of these granules. Acid ribonuclease is one of the enzymes associated with the lysosome particles of the cell cytoplasm. Since these subcellular particles are assuming increasing importance in cellular biology, this communication presents a new technique developed during a study undertaken to demonstrate the localization of this activity by cytochemical methods.

The technique is based on the use of ribonucleic acid (RNA) from yeast as substrate and is a modification of the histochemical technique for intracellular localization of acid-deoxyribonuclease in the rat liver described by Vorbrodt(5). Particular attention has been paid in composing the incubation medium that makes it possible to obtain intracellular localizations of acid RNA-ase activity in conformity with those indicated by quantitative data from isolated subcellular fractions described by Houck & Berman (1).

Materials and methods. Blood smears obtained from the tails of Swiss mice of the Webster strain and air dried for 10-30 min-

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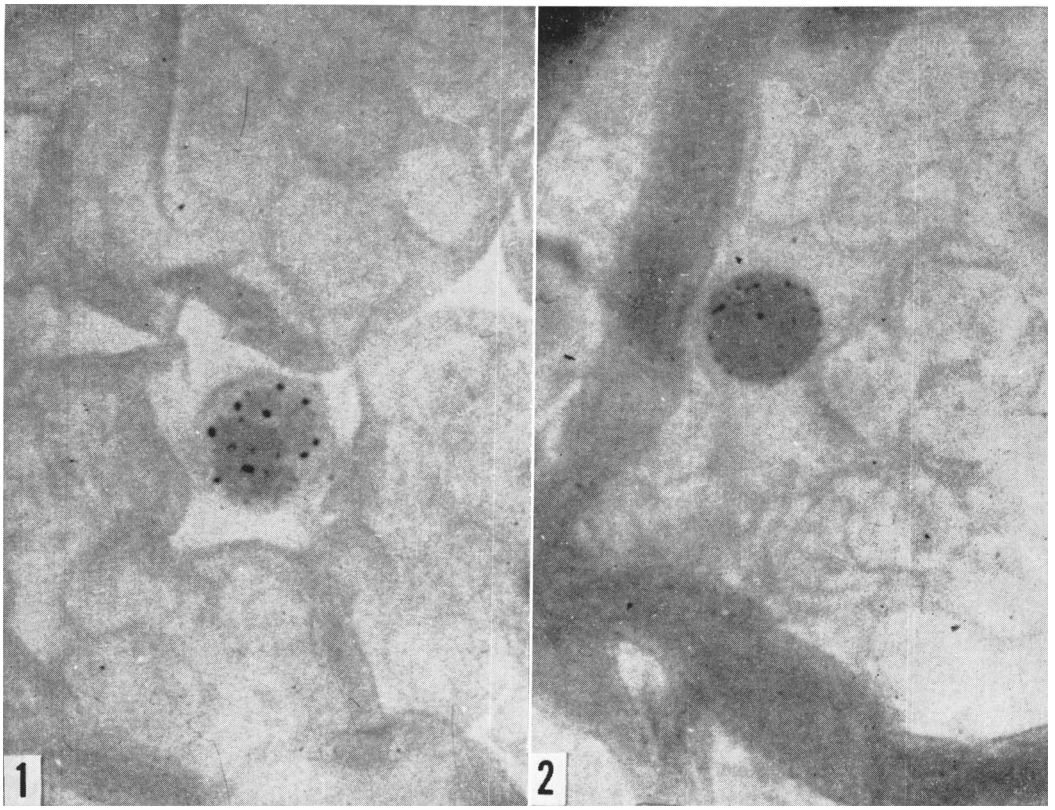

FIG. 1. Polymorphonuclear leucocytes showing sites of enzyme activity marked by dark spots in the perinuclear zone. ×800.

FIG. 2. Lymphocyte showing activity of acid ribonuclease along the thin rim of the cytoplasm and also in the nucleus. ×800.

utes at room temperature were fixed for 10 minutes in formalin vapors. This was accomplished by adding enough 10% formalin (4% formaldehyde) to moisten a thin layer of absorbent cotton at the bottom of a small Coplin jar which was covered with a larger glass jar. Following fixation, the smears were incubated for up to 1½ hours at 37°C. The incubation solution was prepared immediately before use from stock solutions. The composition of the incubation medium was as follows.

RNA (yeast)	15 mg
Acid phosphatase	5 mg
0.2 M Acetate buffer	12.5 ml
0.4 M Lead nitrate	1 ml
Distilled water to 50 ml	

After incubation the smears were washed briefly in distilled water and placed in dilute ammonium sulphide solution for 10 minutes. After washing in distilled water, the smears were counterstained in a one per cent Safranin solution for 30 seconds, rinsed in distilled water, dried and mounted in glycerine jelly.

Results. Microscopic examination reveals the sites of enzyme activity as marked by deposits of dark blue granules. In the neutrophilic series these granules vary in number from 5-15 per cell, distributed mostly in the perinuclear region and along the cell wall (Fig. 1), with the juvenile cells showing higher enzymatic activity than the mature form. There was activity also in the lmphocytes indicated by small dark blue granules in the cytoplasm (Fig. 2). Every cell belonging to the neutrophilic series and lymphocytic series showed some degree of activity. Basophils, eosinophils and monocytes were not definitely identified as having enzymatic activity.

When RNA was omitted from the incubation medium no staining occurred except for the presence of a few faintly staining bluish granules in some neutrophils. On the other hand the addition of sodium fluoride (0.01 M) to the incubation media completely inhibited the reaction.

Discussion. The cytochemical reaction just described is based on a modification of the lead sulphide method devised by Gomori(6) to show acid phosphatase activity. It is presumed that nucleotide released from RNA in the incubation medium by the action of tissue RNA-ase are dephosphorylated by added acid phosphatase. The resulting inorganic phosphate ions are deposited as the lead salt.

Zittle and Reading(7) claimed that the major portion of RNA-ase activity was found in the red blood cells which by our technique did not show any activity. The evidence for enzymatic activity in the leucocytes is consistent with studies made by Dubos and Mecleod(8) who found that polymorphonuclear leucocytes released RNA-ase. Similar findings were made by Houck and Berman(1) and Gupta and Herriot(9). They also established a relationship between enzyme level and white cell count. Cohn and Hirsch(4) described the lysosomal nature of the specific granules of polymorphonuclear leucocytes. They described the presence of most of the lysosomal enzymes like acid phosphatase, ribonuclease, deoxyribonuclease, beta glucoronidase, cathespsin and 5′-nucleotidase in these granules.

During the present study it has been found that relatively few granules in the perinuclear zone of the cytoplasm showed ribonuclease activity. Therefore, it is tempting to conclude that not all the specific granules of the polymorphonuclear leucocytes carry all or most of the enzymes generally associated with lysosomes. In other words they might be different biochemical entities. The question of the extent and nature of heterogeneity among lysosomes was reported by de Duve and his coworkers(10) who have found slight differences among lysosomal enzymes in liver cells. In view of the widespread distribution of acid phosphatase revealed by cytcohemical observations in the leukocytes by Rabinovitch and Andrucci(11) and Goldberg(12), it is unlikely that each enzyme is associated singly with a distinct type of particle although some degree of enzymic heterogeneity in this group of lysosomes in leucocytes could be expected. It might be a likely hypothesis, on the basis of the present findings, that all the specific granules are not lysosomal, except a few, particularly in the perinuclear zone of the cytoplasm, but if they are all lysosomal in nature then they might be different biochemical entities. More cytoenzymological studies are needed to establish a direct link between the biochemical concept of these leucocytic granules and their morphological identification.

Summary. 1. A method of fixation and composition of incubation media for staining acid ribonuclease in the peripheral leucocyte is described. 2. Localization of ribonuclease in the lysosome (specific granules of polymorphonuclear leucocytes) and their possible heterogenic characters are discussed. 3. Since the technique described has reproducible and artifact-free results, it is hoped that it will facilitate investigation of ribonuclease in both blood and bone marrow cells in different pathological conditions.

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1. Houck, J. C., Berman, L. B., *J. Appl. Physiol.,* 1958, v12, 473.

2. Novikoff, A. B., *Biol. Bull.,* 1959, v117, 385.

3. Cohn, Z. A., Hirsch, J. G., *J. Exp. Med.,* 1960, v112, 983.

4. ———, *ibid.,* 1960, v112, 1015.

5. Vorbrodt, A. J., *J. Histochem. and Cytochem.,* 1961, v9, 647.

6. Gomori, G., *ibid.,* 1956, v4, 453.

7. Zittle, C. A., Reading, E. H., *J. Biol. Chem.,* 1945, v160, 519.

8. Dubos, R. J., Mecleod, C. M., PROC. SOC. EXP. BIOL. AND MED., 1937, v36, 696.

9. Gupta, S., Herriot, R. M., *Arch. Biochem.,* 1963, v101, 88.

10. de Duve, C., *Biochem. J.,* 1959, v73, 610.

11. Rabinovitch, M., Andrucci, D., *Blood,* 1949, v4, 580.

12. Goldberg, A. F., Gibor Barka, *Nature,* 1962, v195, 297.

Received December 7, 1963. P.S.E.B.M., 1964, v115,