the assistance of the latter in the design of the vacuum-tube voltmeter used in these studies.

1. Northrop, J. H., *J. Gen. Physiol.*, 1948, v31, 213.
2. Kolthoff, I. M., and Laitinen, H. A., *pH and Electro Titrations*, Second Edition, New York, John Wiley and Sons, Inc., 1941.
3. Van Slyke, D. D., *J. Biol. Chem.*, 1923, v58, 523.
4. Wilson, D. W., and Ball, E. G., *J. Biol. Chem.*, 1928, v78, 1.
5. Eisenman, A. J., *J. Biol. Chem.*, 1929, v82, 411.
6. Bell, M. E., *J. Physiol.*, 1933, v79, 191.
7. Sanders, G. P., *J. Dairy Sci.*, 1939, v22, 841.
8. Ingram, M., and Hawthorne, J. R., *J. Soc. Chem. Ind.* (London), 1945, v64, 196.
9. Cunningham, B., Kirk, P. L., and Brooks, S. C., *J. Biol. Chem.*, 1941, v139, 11.
10. Tremblay, J. L., *Naturaliste can.*, 1936, v63, 269.
11. Ingram, M., and Bryan, J. M., *J. Soc. Chem. Ind.*, (London), 1948, v67, 359.
12. Sunderman, F. W., and Williams, P., *J. Biol. Chem.*, 1933, v102, 279.
13. Wu, H., *J. Biol. Chem.*, 1922, v51, 21.

Received September 30, 1952. P.S.E.B.M., 1952, v81.

## Plasmal Reaction in the Blood and Hemopoietic Organs. (19869)

IVAN MOTA, SAKAE YONEDA, AND IRIA RABINOVITCH. (Introduced by L. C. Junqueira.)
*From the Laboratory for Cell Physiology, Fac. de Medicina, S. Paulo, Brasil.*

Although the occurrence of the plasmal reaction of Feulgen and Voit(1) has been observed in many tissues and cells, no reference has been found in the literature concerning its presence in the cells of the blood and hemopoietic organs.

*Material and methods.* Ten adult normal rats of the Wistar strain were killed by a blow on the head. Smears of blood, bone-marrow, thymus, spleen and lymphatic glands were made and immediately immersed (before drying) in a mixture of equal parts of Schiff's reagent and saturated $HgCl_2$ solution during one minute, and then passed through 2 sulfurous acid baths of 5 minutes each and then rinsed with distilled water. Control smears were immersed in Schiff's reagent diluted with an equal part of distilled water for the same period, and afterwards treated as above described. Counterstaining with diluted methyl green solution was performed in some slides in order to help the identification of the cells. Smears from blood and sternal bone marrow obtained from 3 patients without hematological disorders were similarly treated. A positive reaction is indicated by a red stain present in the test slides and absent in the controls.

*Results.* As human and rat blood and bone marrow cells presented a similar reaction, the following data apply to both.

a) *Blood cells.* A positive reaction was observed in the cytoplasm of neutrophilic and eosinophilic granulocytes, monocytes and in the platelets. Reaction of neutrophils and monocytes was diffuse throughout the cytoplasm; eosinophils presented a less intense staining, localized between the generally unstained granules. Due to the small number of basophils no observations on them are reported. A positive reaction could be observed in the majority of the lymphocytes, sometimes diffuse throughout the cytoplasm and occasionally localized in granules. Red blood cells were always negative.

b) *Bone marrow cells.* The reaction was intensely positive in the cytoplasm of the

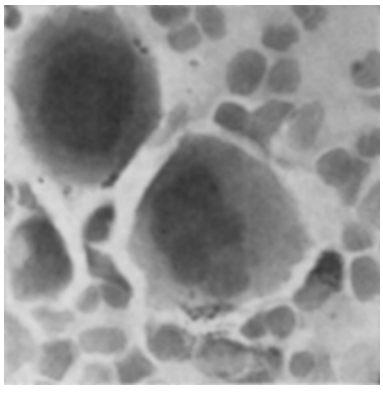

FIG. 1. Positive plasmal reaction in the cytoplasm of megakaryocytes and eosinophils. Nuclear counterstaining with methyl green. Approx. × 1400.

megakaryocytes. A weaker but undoubtedly positive reaction was observed in the cytoplasm of neutrophilic and eosinophilic myelocytes, plasmacytes and reticular cells. In the human material, cells which could be recognized as proerythroblasts and basophilic erythroblasts presented a juxtanuclear positively reacting zone. More mature forms did not stain.

c) *Thymus and lymphatic glands.* A positive reaction was observed only in the plasmacytes and reticular cells. Lymphocytes were always negative in these organs. In the plasmacytes the reaction was localized in a juxtanuclear zone, diffusely or in granules. Granulocytes when present reacted positively.

d) *Spleen.* A red stain denoting a positive reaction could be observed in the megakaryocytes, neutrophilic and eosinophilic granulocytes, macrophages, plasmacytes and reticular cells. These cells stained similarly as the above described ones.

*Discussion.* The positive plasmal reaction observed indicates the presence of plasmalogens (glyceryl-phosphoryl-ethanolamine and possibly analogous compounds (Lovern)(2) in the blood and hemopoietic organ cells. The positivity of the reaction in the cytoplasm of the megakaryocytes and in the platelets, adds more cytochemical evidence to the megakaryocytic origin of the platelets. The physiological significance of the presence of these compounds in the cells is unknown, but perhaps it might be connected with a protective mechanism(3). This reaction has been applied to pathological human material and a detailed account of the results obtained will be published elsewhere.

*Summary.* A positive cytoplasmic reaction was obtained by a modified technic of plasmal reaction in the following cells from human and rat blood and hemopoietic organs: megakaryocytes, platelets, neutrophils, eosinophils, lymphocytes, monocytes, plasmacytes, early erythroblasts and reticular cells.

Thanks are due to Prof. Junqueira for his helpful suggestions.

1. Feulgen, R., and Voit, K., *Pflügers Arch. Physiol.*, 1924, v206, 389.

2. Lovern, J. A., *Nature*, 1952, v169, 969.

3. Oster, K. A., and Mulinos, M. G., *J. Pharm. Exp. Therap.*, 1944, v80, 132.

Received July 22, 1952. P.S.E.B.M., 1952, v81.

## Effect of Protamine Sulfate in Neutralizing Coagulation Defect Produced by Paritol.* (19870)

LLOYD G. BARTHOLOMEW AND NELSON W. BARKER. (Introduced by Frank D. Mann.)
*From the Division of Medicine, Mayo Clinic, Rochester, Minn.*

In the constant search for an ideal anticoagulant, many new compounds with potent anticoagulant ability have been tried clinically. One of these new compounds is paritol, a polysulfuric acid ester of polyanhydromannuronic acid. Its mode of action has not been definitely determined but it is thought to be similar to that of heparin. Animal experiments(5) have suggested the presence of an antithrombin inhibitor in the peripheral blood as the natural inactivator of paritol.

The use of powerful anticoagulants always carries the possibility of hemorrhage. In view of this it is desirable to have an adequate neutralizing agent which will assure some degree of safety from hemorrhage. Protamine sulfate has been used as an antagonist to the coagulation defect produced by heparin. It seemed advisable to determine the extent of this neutralizing effect on the coagulation defect produced by paritol. Protamine sulfate was found(4) to have a clear-cut neutralizing effect upon paritol activity in dogs and rab-

* Abridgement of thesis submitted by Dr. Bartholomew to the Faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Master of Science in Medicine.