by the DNA of the spleen after standard hemorrhage or anoxic anoxia. Carnot(4) first postulated a humoral mechanism for regulation of erythropoiesis. It is generally accepted that in the plasma of erythropoietically stimulated animals there is a factor that will increase red cell formation in normal animals. Using a different method here described, the presence of such a factor in the plasma of phenylhydrazine anemic or hypoxic rabbits has been confirmed.

Summary. 1. A method for evaluating erythropoietic stimulation has been described. 2. Following a standard hemorrhage there is a 2- to 5-fold increase in P^{32} uptake by splenic DNA. 3. Exposure to anoxic anoxia increases P^{32} uptake by the DNA of spleen. 4. Injection of anemic rabbit or hypoxic rabbit plasma increased P^{32} uptake by splenic DNA in normal receptor rats.

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Relation Between Platelet Integrity and Sulfhydryl Groups.* (24581)

J. L. KOPPEL, LILLIAN V. NOVAK AND JOHN H. OLWIN

Dept. of Surgery, Presbyterian-St. Luke's Hospital, affiliated with College of Medicine, University of Illinois, Chicago

During the last few years platelets have been shown to contain a number of "factors" which are concerned with different phases of clotting mechanism(1). Since it is known that, in formation of thrombin, platelets together with their plasma cofactors produce a powerful intrinsic thromboplastin, it has been postulated that intravascular coagulation may be initiated once appreciable amounts of certain materials are derived from platelets(2). Whether or not release of platelet factors requires actual breakdown of platelets or only changes in their "membrane" permeability characteristics is not as yet known. What is known is that retraction of the fibrin gel requires presence of intact platelets(3) whereas antiheparin and antifibrinolytic activities of platelets are enhanced markedly as result of lysis(4,5). Furthermore, bleeding tendency associated with thrombocytopathy A is related to functional platelet abnormality resulting in increased resistance to disintegration(6). It would thus appear that variations in rate of normal platelet breakdown may have important bearing on several aspects of coagulation mechanism. This paper describes experimental findings suggesting a relationship between platelet integrity and platelet sulfhydryl groups.

Methods and materials. Platelet suspensions were prepared from fresh human blood collected with versene (ethylenediamine tetraacetate Na_2-salt) according to the method of Minor and Burnett(7). Platelets were washed 3 times in physiological saline containing 0.1% versene and 2% Triton WR-1339 (Rohm & Haas), the latter substance used to facilitate resuspension following centrifugation. They were finally suspended in physiological saline containing 2% Triton WR-1339 to give "standard suspension" containing approximately 2.5 x 10^6 platelets/mm^3. Sulfhydryl inhibitors, studied for effects on platelet stability, were dissolved in de-ionized distilled water, adjusted to pH 6.5, and added to "standard platelet suspension" in small volumes to preserve isotonicity. Reduced glutathione and cysteine ethyl ester were dissolved in de-ionized distilled water immediately prior to use, and adjusted to pH

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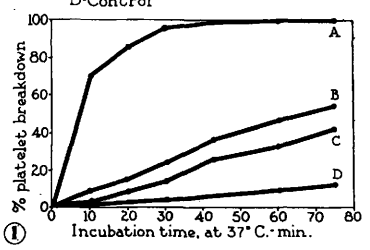
6.5. Samples in which equal volume of physiological saline replaced the test substances served as control. The extent of platelet breakdown was determined by direct count according to the method of Rees and Ecker (8). All counts were carried out in duplicate, the standard error of method being ± 11%. All experiments were carried out at least in triplicate and the results reported are averages. All glassware in the collection of blood, in preparation of platelet concentrates and in experiments was silicone-coated (G. E. Dri Film #9977). Metal surfaces were treated with Arquad(9). Reduced glutathione, N-ethyl maleimide and cysteine ethyl ester were obtained from Schwarz Lab., Na-iodoacetate and alloxan were Eastman Kodak products, p-chloromercuribenzoic acid and iodosobenzoic acid were obtained from Sigma Chemical Co., Salyrgan from Winthrop-Stearns, and phenyl mercuric hydroxide from Matheson, Coleman and Bell. All other chemicals used were of standard reagent grade.

Results. Freshly prepared human platelets were incubated with phenyl mercuric hydroxide, a mercaptide-forming agent known to inhibit many SH-dependent enzymes. Platelet destruction occurred rapidly, although in presence of SH-containing substances, such as reduced glutathione or cysteine ethyl ester, its rate was markedly decreased (Fig. 1). Findings similar to these have been reported for sheep erythrocytes by Benesch and Benesch (10) and for human erythrocytes by Sheets, Hamilton and DeGowin(11).

Effect of Sulfhydryl-Containing Substances on Rate of Platelet Breakdown in Presence of Phenylmercuric Hydroxide

A- 1.0 x 10^{-5} M phenylmercuric hydroxide
B- 1.0 x 10^{-5} M phenylmercuric hydroxide plus 1.0 x 10^{-4} M cysteine ethyl ester
C- 1.0 x 10^{-5} M phenylmercuric hydroxide plus 1.0 x 10^{-4} M reduced glutathione
D- Control



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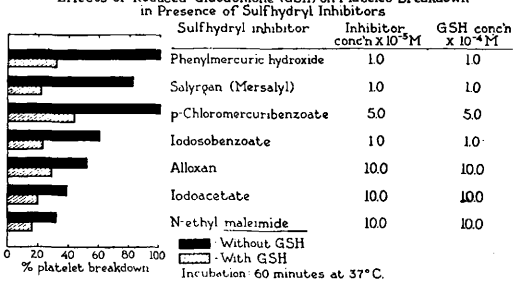
Effects of Reduced Glutathione (GSH) on Platelet Breakdown in Presence of Sulfhydryl Inhibitors



②

FIG. 1 and 2.

Results obtained with various other sulfhydryl inhibitors, both in presence and absence of reduced glutathione, are presented in Fig. 2. Regardless of type of reaction which these substances undergo with sulfhydryl groups, an increased rate of platelet breakdown is observed not only with mercaptide-forming agents, but also with alkylating substances such as iodoacetate and sulfhydryl-oxidizing agents such as iodosobenzoate. In all cases studied the effects observed were highly sensitive to concentration and could always be decreased in extent by suitable concentrations of SH-containing substances such as cysteine or reduced glutathione. The results seem to point towards a relationship between platelet sulfhydryl groups on one hand and the maintenance of platelet integrity on the other.

Discussion. Of particular interest are the results obtained with N-ethyl maleimide. This substance which is highly specific in its ability to react with sulfhydryl groups, brought about a significant breakdown of platelets in concentration of 10^{-4} M. On the other hand, it has been reported that the same material in concentration of 5 x 10^{-4} M does not hemolyze human red cells, although other sulfhydryl reagents do(12). An explanation for the difference between these 2 sets of observations is not immediately apparent, although it may be suggested that interstices of the platelet membrane are larger than those of the red cell, thereby allowing access of N-ethyl maleimide to the critical SH-groups of the intact platelet.

It has been previously reported that whole platelets contain a number of active dehydrogenase systems(13,14) and that activities of some of these enzymes can be materially increased by sulfhydryl-containing substances (13). As it is becoming increasingly evident

that platelets do carry out various metabolic activities, one may wonder about the interrelationship between their rate of metabolism and their ability to maintain their structural integrity. Is a normal rate of metabolism a prerequisite for an intact platelet structure or is the maintenance of an intact structure dependent on normal rate of metabolism? At present there is no clear-cut answer to these questions although data on effects of various other types of enzyme inhibitors on platelet stability indicate that it is more likely that platelet structure is dependent on platelet metabolism (unpublished). Thus, it is suggested, although by no means proved, that increased rate of platelet breakdown observed in presence of SH-inhibitors may be brought about by effects of these substances on a variety of SH-dependent enzyme systems essential to normal platelet metabolism.

Summary. 1. Incubation of freshly prepared human platelets with a variety of sulfhydryl inhibitors results in increased rate of platelet breakdown. 2. In all cases studied the effects of inhibitors can be decreased in extent by suitable concentrations of SH-containing substances such as cysteine or reduced glutathione. 3. Results obtained suggest a direct relationship between platelet sulfhydryl groups and platelet structure. The possible link of this relationship to platelet metabolism is briefly discussed.

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Cytopathogenic Effect Produced by Polyoma Virus in Cultures of Milk-Adapted Murine Lymphoma Cells (Strain P388 D_1).* (24582)

A. S. RABSON AND F. Y. LEGALLAIS (Introduced by Harold L. Stewart)
Laboratory of Pathology, Natl. Cancer Inst., N.I.H., Bethesda, Md.

Production of pleomorphic tumors of the parotid glands of mice by inoculation of newborn animals with cell-free filtrates prepared from mouse neoplasms has been described both by Gross(1) and by Stewart,(2,3) and has been confirmed in several laboratories(4,5, 6). In addition to parotid gland tumors, the inoculated mice have often developed tumors in other salivary glands, mammary glands, the thymus gland, the subcutaneous tissue and other sites. Recently, Eddy and her associates have reported that preparations of the agent also produce sarcomatous and angiomatous tumors in hamsters(7). Evidence suggesting replication of the agent in monkey kidney cell cultures was obtained, but cytopathogenic changes were not observed in most of the infected cultures(8). Subsequently, it was shown that the agent could be propagated in minced mouse embryo cultures, and cytopathogenic changes were observed with some preparations(9). Recently, Eddy and her as-

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