Communications

Activation of Purified Prothrombin in Association with Synthetic Organic Compounds and Platelet Extract[1]

In the clotting of blood, fibrinogen is transformed to fibrin by means of the action of thrombin. The chemical reactions that occur when thrombin arises from prothrombin are not fully understood. Attempts are being made to elucidate the complex problem by isolating and studying purified prothrombin. Efforts are also being made to obtain the substances that participate in the activation of prothrombin in purified form.

When prothrombin preparations that contain Ac-globulin are mixed with calcium, platelet extracts, and a partially purified platelet cofactor, thrombin forms rapidly (*1*). The platelet cofactor, which we obtain from plasma in partially purified form, is considered to be the antihemophilic factor. We have discovered that certain synthetic organic compounds can substitute for it in the activation of purified prothrombin. If there are other known instances where an organic compound can substitute functionally for a protein, they must be rare. We are not aware of any comparable observation. Our finding also has implications related to the hemophilia problem on the basis that the search for therapeutic agents is encouraged. Moreover, technical difficulties are greatly reduced for studying the role of platelet derivatives in the activation of prothrombin.

Our studies have been extensive, but as an illustration of this work Linadryl (4-(2-(benzhydryloxy) ethyl) morpholine) serves as a typical example. A purified prothrombin preparation purposely high in Ac-globulin was made from bovine sources by methods previously described (*2*). The following activation mixture was made in the order stated: 1.0 ml purified prothrombin containing 3000 units/ml plus 0.5 ml of 0.153 *M* $CaCl_2$ dissolved in imidazole buffer (*3*), plus 0.5 ml of 0.1% Linadryl solution plus 0.5 ml platelet extract prepared as previously described (*1*). To this was added purified thrombin to give only 10 units/ml in the reaction mixture. The purified thrombin was obtained from purified prothrombin by activating the latter in 25% sodium citrate solution (*4*). Within 15 to 45 min the prothrombin transforms to thrombin. If thrombin is not added to the activation mixture the transformation of prothrombin to thrombin may not result in a full yield of thrombin. It seems likely here that thrombin is acting by its well-known effect on Ac-globulin. Linadryl is one of the compounds of the antihistamine group. We are continuing our studies with other related compounds. Benadryl (2-benzhydryloxy-N,N-dimethylethylamine HCl), Decapryn succinate (2-(a-(2-dimethylamino-ethoxy)-amethybenzyl) pyridine succinate), and Phenindamine (2-methyl-9-phenyl-2,3,4,9-tetrahydro-1-pyridindene hydrogen tartrate) are also effective. Histamine itself can also be used.

[1] Supported by research grant H-1467 from the National Heart Institute of the National Institutes of Health, Public Health Service.

Marvin Murray
Shirley A. Johnson
Walter H. Seegers
Department of Physiology and Pharmacology
Wayne University College of Medicine
Detroit, Michigan

References

1. Johnson, S. A., Smathers, W. M., and Schneider, C. L. *Am. J. Physiol.* **170**, 631 (1952).
2. Seegers, W. H., Loomis, E. C., and Vandenbelt, J. M. *Arch. Biochem.* **6**, 85 (1945).
3. Mertz, E. T., and Owen, C. A. *Proc. Soc. Exptl. Biol. Med.* **43**, 204 (1940).
4. Seegers, W. H., McClaughry, R. I., and Andrews, E. B. *Ibid.* **75**, 714 (1950).

Received December 12, 1953.

An Open Letter to the Assistant Secretary for Research and Development, Department of Defense

I wish to bring to your personal attention a defect in the administration of the rule, based upon existing law, which requires certification, by a designated officer of the armed forces, that individuals working on secret parts of a government research contract are acceptable for access to secret security information in the custody of the contractor. It also suggests a partial remedy for this defect. The intent of the rule is, clearly, to make available to the contractor the services of trustworthy persons. The inordinate delay in such certifications which results under present conditions from even the most trivial facts or rumors turned up in routine investigations is especially crippling to research projects where the people of most value are not nearly interchangeable parts, like machine operators in a factory, and where the time available under a contract may be relatively short.

The situation objected to in the preceding paragraph has been forcibly brought to my attention as director of an urgent defense project supported since January 1951 under Contracts N7onr 288(09) and Nonr 609(02) between Yale University and the Office of Naval Research. This project has been in force for more than three years, but its continuance has had to be negotiated repeatedly, and its life expectancy is not now and never has been as long as a year. The Director of the New York Branch Office of Naval Research is the officer who must, in this case, decide as to secret clearances. He bases his decision upon a Personal Security Questionnaire, filled out by the individual and forwarded by the contractor, here Yale University, and upon such additional information as he can get from the Naval Intelligence Officer of the Third Naval District, also in New York City. Most of any detailed investigation of the individual's history, suggested by