Meetings

Blood Clotting: Enzymic Activation

Blood clotting is basically the polymerization of the plasma protein "sol" fibrinogen to a colloidal fibrillar "gel" fibrin, under the enzymatic influence of thrombin. Thrombin is derived from an inactive precursor (plasma) protein, prothrombin. Special enzymic mechanisms concerned with the activation of this prothrombin were discussed at a symposium held on 17 April during the Chicago meetings of the Federation of American Societies for Experimental Biology.

An "enzymic" phenomenon (crudely analogous to the activation of pancreatic trypsinogen by enterokinase) was suggested at the turn of the century by the European (Morawitz) term "thrombokinase." The first speaker at the symposium, J. H. Milstone (Yale University) reviewed his past and present work on the preparation of a precursor "prothrombokinase" and active thrombokinase from plasma. While thrombin formation can be markedly increased by additions of factor V, phospholipid (cephalin), and ionized calcium, Milstone presented evidence to suggest that, with strong prothrombin and enzyme, thrombokinase serves as a "direct" activator of prothrombin. The enzyme, however, can work in nanogram amounts, and it is under these conditions that the other clotting factors assume the greatest significance.

T. H. Spaet (Montefiore Hospital, New York) approached the topic from the point of view of "intermediate" reactions of clotting, particularly of reactions that precede the conversion of prothrombin to thrombin. This approach was especially advanced by the intrinsic "thromboplastin" generation test of Biggs and Douglas (1953), in R. G. Macfarlane's laboratory (Oxford), and by the discovery of many new factors participating in blood coagulation. In 1956 Bergsagel and Hougie provided evidence concerning certain intermediates, including "product I," which have been much studied in Europe and elsewhere. Spaet and his colleagues have worked on the purification of product I, and Spaet stated that product I appears to be an enzyme derived from the activation of "factor X," which thereby has come to be considered a key factor in the conversion of prothrombin to thrombin.

W. H. Seegers (Wayne State University) has been working on the purification of prothrombin (and its derivatives) and of other clotting factors since 1940. He urged reorientation of scientific thinking concerning the blood-clotting mechanism around prothrombin and its various derivatives. These derivates, according to experiments with highly purified prothrombin, are not only thrombin but also an important series of other agents (by-products) or "autoprothrombins." The appearance of such "autoprothrombins" depends upon the composition of the mixtures with the parent prothrombin. According to Seegers, prothrombin in 25 percent sodium citrate yields thrombin and another enzyme, "autoprothrombin C." When prothrombin is incubated with thrombin alone, it yields "autoprothrombin II," but in the presence of calcium ions and "Ac-globulin" (factor V), it yields new thrombin and "autoprothrombin III." If prothrombin is treated with autoprothrombin C alone, it yields "autoprothrombin I_c," whereas, in the presence of Ca^{++} and V, it yields new thrombin and autoprothrombin III (as in the corresponding experiment with thrombin). Seegers's new idea is that certain clotting factors (VII, X, and autoprothrombin C), in addition to thrombin, do not exist as such in the blood plasma but are derived from prothrombin during the natural clotting processes, once these are initiated in vitro or (pathologically) in vivo. He therefore considers factor VII or factor X deficiencies to be, in reality, the result of some abnormality of the prothrombin. Thrombin and autoprothrombin C are thought to be enzymatic activators, not only of the completion of thrombin formation (and hence of the fibrin clotting), but also of the "autoprothrombin" by-products. It is to these "autoprothrombins" (or prothrombin derivatives) that Seegers ascribes many of the "activities" in coagulation tests, which have previously been regarded as activities of independent clotting factors.

The symposium chairman, J. H. Ferguson (University of North Carolina) approached the topic from the point of view of his long-held concept of "thromboplastic enzymes." These were originally defined as dependent upon specific phospholipids (*"pro*thromboplastic") and Ca^{++}, and were later shown to require, also, certain plasmatic cofactors (V, VII, or X, depending on the particular enzyme). Ferguson presented his method of quantitating thrombin yields in an old-type "two-stage" thrombin generation test. For this it is not necessary to have highly purified prothrombin, provided the tests provide a means of identifying the significant factors present, even in very small quantities. Using trypsin, stypven (Russell's viper venom), and the procoagulants (thrombokinase, product I, and autoprothrombin C) discussed by the previous speakers, Ferguson showed quantitatively their dependence on Ca^{++}, lipid, and factor V in the thrombin-forming test system. Tissue thromboplastin (human brain extract) and a new urinary procoagulant (von Kaulla) behaved differently.

Next there was a review of the phospholipids (including precise bioassay of their "prothromboplastic" activities and other properties, especially relating to pure platelet phosphatidyl serine and phosphatidyl-ethanolamine); of factor V; and of enzyme relationships to factor VII and factor X activities. It was concluded that the "thromboplastic enzyme" idea has been a useful working hypothesis but may now require some modification, in view of the experiments with factor VII and factor X. The new procoagulants from blood do not need these clotting factors, nor do they need factors VIII, IX, XI, and XII, but they do require V, Ca^{++}, and phospholipid. They all appear to be very similar, perhaps identical. For the reason giv-

en, these enzymes (or this enzyme) can bypass many clotting-factor deficiencies. Whether this may have practical application in the therapy of coagulation disorders is a matter for future investigation.

JOHN H. FERGUSON
University of North Carolina, Chapel Hill

Forthcoming Events

June

2–3. **Photovoltaic Specialists**, 4th annual conf., Cleveland, Ohio. (P. Rappaport, RCA Laboratories, Princeton, N.J.)

2–4. **Global Communications**, intern. symp. (Globcom VI), Philadelphia, Pa. (R. Guenther, RCA Communications Systems Div., Bldg. 1-3-1, Camden, N.J.)

2–4. **Telemetering**, natl. conf., Los Angeles, Calif. (W. S. Pope, 8420 Quinn St., Downey, Calif.)

2–5. **Food Microbiology**, 4th intern. symp., Göteborg, Sweden. (N. Molin, Swedish Inst. for Food Preservation Research, Göteborg 16)

2–6. **Acoustical** Conf., 3rd., Budapest, Hungary. (Acoustics Div., Hungarian Soc. of Optics, Acoustics, and Film Techniques, Szabadság tér 17, Budapest 5)

2–6. **Opthalmic-Optics**, intern. congr., Copenhagen, Denmark. (Danmark Special Optiker-Forening, Vesterbrogade 41B, Copenhagen 5)

3–5. Collaborative **Pesticides** Analytical Committee, 8th, Wageningen, Netherlands. (R. de B. Ashworth, c/o Plant Pathology Laboratory, Hatching Green, Harpenden, Hertfordshire, England)

3–10. American **Metalworking Technology** for the European Community (AMTEC), Brussels, Belgium. (E. L. Koester, ASTM, 10700 Puritan Ave., Detroit, Mich.)

7–9. National Public Relations Council of **Health and Welfare Services**, New York, N.Y. (The Council, 257 Park Ave. S., New York 10010)

7–9. **Isotopically Labeled Drugs** in Experimental Pharmacology, conf., Chicago, Ill. (L. J. Roth, Dept. of Pharmacology, Univ. of Chicago, Chicago 60637)

7–11. **Special Libraries** Assoc., St. Louis, Mo. (Mrs. J. North, Missile and Space Div., Lockheed Aircraft Corp., Palo Alto, Calif.)

7–12. **Mass Spectrometry** and Allied Topics, 12th annual conf., Montreal, Quebec, Canada. (N. D. Coggeshall, Gulf Research and Development Co., P.O. Drawer 2038, Pittsburgh, Pa. 15230)

7–13. European **Ophthalmological** Soc., 2nd congr., Vienna, Austria. (J. François, 15, Place de Smet de Naeyer, Ghent, Belgium)

8–9. Basic **Cancer Research**, 2nd Scandinavian symp., Stockholm, Sweden. (K. E. Hellström, c/o Riksföreningen mot Cancer, Postgiro 90 19 51, Stockholm)

8–10. **Quasi-Optics**, symp., Polytechnic Inst. of Brooklyn, 14th, New York, N.Y. (Polytechnic Inst. of Brooklyn, 55 Johnson St., Brooklyn 1)

8–11. **Cardiovascular** Conf., 2nd intern., St. Adele, Quebec, Canada. (D. F. M. Bunce, Dept. of Physiology, College of Osteopathic Medicine and Surgery, Des Moines, Iowa)

8–11. International **Planned Parenthood Federation**, conf. of region for Europe, Near East, and Africa, London, England. (J. Bettie, 6 Pembroke Rd., London, W.1)

8–12. **Surface Contamination**, intern. symp., Gatlinburg, Tenn. (B. R. Fish, Health Physics Div., Oak Ridge Natl. Laboratory, P.O. Box X, Oak Ridge, Tenn.)

9. International Assoc. for the **Prevention of Blindness**, Vienna, Austria. (J. P. Baillart, 47, rue de Bellechasse, Paris 7°, France)

9–11. **Cobalt Applications**, intern. meeting, Brussels, Belgium. (Cobalt Information Center, Battelle Memorial Inst., 505 King Ave., Columbus 1, Ohio)

9–11. **Electromagnetic Compatibility**, 6th natl. symp., Los Angeles, Calif. (J. A. Eckert, Dept. 3441/32, Northrop Norair, 3901 West Broadway, Hawthorne, Calif.)

9–12. Canadian Federation of **Biological Societies**, Halifax, N.S. (A. H. Neufeld, The Federation, Univ. of Western Ontario, London, Ont., Canada)

9–12. **Max Planck Soc. for the Furtherance of Science**, general meeting, Hamburg, Germany. (Max-Planck Gesellschaft zur Förderung des Wissenschaften e.V., Düsseldorf, Germany)

10–12. **Heat Transfer and Fluid Mechanics**, Berkeley, Calif., (S. Levy, General Electric Co., 150 Curtner Ave., San Jose, Calif.)

10–19. **Intergovernmental Oceanographic Commission**, 3rd session, Paris, France. (W. S. Wooster, Office of Oceanography, UNESCO, Place de Fontenoy, Paris 7°)

11–13. **Manufacturing Chemists'** Assoc., 92nd annual, White Sulphur Springs, W. Va. (MCA, 1825 Connecticut Ave., NW, Washington, D.C.)

11–13. **Population** Assoc. of America, San Francisco, Calif. (P. C. Glick, Bureau of Census, Washington, D.C. 20233)

13–19. **Medical Film Festival**, Helsinki, Finland. (W. M. A.-Film Finmedicas, Ullanlinnankatu 1, Helsinki)

13–19. **World Medical Assoc.**, 18th general assembly, Helsinki, Finland. (H. S. Gear, 10 Columbus Circle, New York, N.Y. 10019)

14–17. American Assoc. of **Feed Microscopists**, 12th annual, Hot Springs, Ark. (G. M. Barnhart, Missouri Dept. of Agriculture, State Office Bldg., Jefferson City)

14–17. American **Nuclear** Soc., 10th annual, Philadelphia, Pa. (O. J. DuTemple, 244 E. Ogden Ave., Hinsdale, Ill. 60502)

14–18. **Industrial Pharmaceutical Research**, 6th natl. conf., Land O'Lakes, Wis. (L. W. Busse, 190 Pharmacy Bldg., Univ. of Wisconsin, Madison 6)

14–18. **Health Physics** Soc., 9th annual, Cincinnati, Ohio. (H. F. Kolde, Taft Sanitary Engineering Center, Cincinnati)

14–19. **Alpha Chi Sigma** Fraternity, Greenvale, L.I., N.Y. (M. L. Griffin, 5503 E. Washington St., Indianapolis, Ind.)

14–19. **Cardiology**, 7th inter-American congr., Montreal, P.Q., Canada. (The Congress, 2052 St. Catherine St., W., Montreal 25)