That my colleagues and myself within that time have accomplished a few things relative to the teaching of plant physiology and along the line of physiological investigations, we believe no one will deny. We can say truthfully and in all sincerity that no rivalry whatsoever relative to the subject-matter to be investigated exists between the various departments of this institution. We talk without restraint to various members of other departments and ask their advice about work that we are doing. They in turn are free to consult us relative to matters in which they are engaged.

Recently one of our students who had finished his undergraduate work at this institution went to a neighboring university to see about taking graduate work leading to the doctorate. He found, to his consternation, that the botany and the chemistry departments of the institution were at swords' points because each felt that certain members of the other department were transgressing upon their sacred domain. This graduate student came back to our institution a sadder but wiser man. He had not realized until that time that such bitter rivalries exist within educational institutions.

There is unquestionably sufficient truth in any field to satisfy the most arduous workers of all parties. The liquor of our own vintage unquestionably is good but if we should mix the drinks from all sources, we would truly have the real nectar of the gods. We are not a prophet or the son of a prophet, but we predict that unless we quit fussing over tweedledum and tweedledee, the fate of our investigations is sealed, for the public that furnishes us the funds with which to conduct our research will withhold them from those who can not conduct investigations in a cooperative way.

Another fact that has been impressed upon me during forty years of experience is that one may know all about a certain subject and yet be a miserable failure as a teacher. He may be considered a "nut" and no more by his fellow men. We are convinced that to succeed as a teacher, not only in plant physiology, but in any field, two prerequisites are necessary. (a) He must know his subject and keep informed concerning its progress at all times, but (b) he should have common sense. We have frequently been taken to task because we have preached and advocated the last-named characteristics as a prerequisite of success. So far, we have been subjected to no arguments that have in any way whatsoever changed our idea relative to the matter. We would list under the term "common sense" three main factors that we believe make up the meaning of that word: A sense of humor, a knowledge of human nature and a trustfulness in humanity. If any individual possesses these three characteristics to a marked degree and if he, in addition, has a thorough knowledge of his subject, he will succeed as a teacher in his chosen field.

The observations and suggestions that have been listed in this paper are a few that have impressed us in our experience in the field of plant physiology during our forty years of experience in that realm. They are not new thoughts and they have been preached by many from the time that investigational work had its origin. We are convinced that the impressions gathered during our years of experience, if followed even to a limited extent, will benefit both the investigator and the field of plant physiology.

A NEW BLOOD-CLOTTING THEORY

By Dr. JOHN H. FERGUSON

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THE difficult subject of blood coagulation has been so beset by inadequacies of the numerous and often conflicting theories, in the light of experimental fact, that the author has labored for nearly a decade with an experimental approach and great hesitancy in propounding a comprehensive theory. Despite the complexities encountered and many "loose-ends" still to be brought into line, current interest in the preparation of plasma and hemostatics for war use makes the time ripe for presentation of a "working hypothesis" for the stimulation of continued research and, particularly, for the guidance of the many whose interest is at present confronted by the sad lack of agreement among so-called "experts" in the field. The experimental basis for the views here presented is to be found in the author's contributions and reviews, in such coagulation reviews as those in *Ergebnisse der Physiologie* (Morawitz, 1905; Wöhlisch, 1929; 1940), in the chapters on proteolytic enzymes and "thrombase" in Oppenheimer's "Die Fermente und ihre Wirkungen" and in Northrop's "Crystalline Enzymes."

It has long been agreed that the essential feature of blood coagulation is the (specific) conversion of a plasma protein fraction (fibrinogen) from the state of a colloidal hydrosol to that of the corresponding quasicrystalline (oriented micelle) gel (fibrin), and that this is the second of a two-phase process, the first being the elaboration of an essential agent (thrombin) from an inactive, probably protein, pre-

cursor (prothrombin), now known to be also a plasma-, rather than a cell-, constituent.

It has generally been recognized that the normal maintenance of the fluidity of the circulating blood is due to the absence of active thrombin. Since the blood, even the plasma devoid of all formed elements, can be shown experimentally to be a source of all the known factors (prothrombin, ionizable Ca, cephalin, etc.) required for thrombin formation, the problem has been why the necessary reactions do not normally occur in fluid blood.

Morawitz's name is particularly connected with the theory of lack of specific "activators" (of prothrombin), and his term "thrombokinase" and the almost identical term "thromboplastin" (Howell) have continued as working names ("shop numbers," if you will) for their designation. Bordet added some new facts and a nomenclature which only complicated the issues. Howell (Wooldridge, Zak and Bordet said "lecithin") identified the phospholipid *cephalin* as a chemical component of "thromboplastin," but, significantly, went on to develop the rival theory, *viz.*, that the failure of thrombin formation was due to specific "inhibitors." An important outcome of this work (with his pupils McLean and Holt) was Howell's second chemical discovery, "heparin," together with firm foundations for the more recent researches as to its mode of action. The simplicity of the Howell theory has had wide appeal, but "coagulationists" have raised many experimental objections. The first is a definite suspicion that the heparin demonstrable in blood is an artefact of the mode of separation or demonstration. A second is that no satisfactory explanation is forthcoming of the mode of removal of the heparin-type inhibitors in clotting blood. Howell, himself, is forced back to a theory of "neutralization" by "thromboplastin." So, in a roundabout way, both theories come back to this ill-defined agent which is undoubtedly not merely a cephalin-protein compound.

Alex. Schmidt, the father of the "thrombin theory," proceeded from the assumption that blood coagulation (like that of milk) was essentially a protein-enzyme reaction. Nolf and others contributed much to an enzyme view which was fraught with the fallacy that thrombin is, itself, a proteolytic enzyme. We believe this to be fallacious because the purest and most potent thrombins are quite (or almost) free from the ability to produce fibrinolysis and more complete proteolysis (*e.g.*, positive biuret and ninhydrin tests, increase in –COOH (formol titration) or in amino-N). Trypsin will digest, but will not clot, prothrombin-free fibrinogen. One proteolytic enzyme, papain, will do both.

A vast amount of work, with very little direct bearing on blood coagulation, had as its starting-point the "Abderhalden reaction," *i.e.*, the alleged appearance in blood serum of proteolytic enzymes, specific for parenterally injected ("foreign") proteins. The positive evidence for such phenomena has continued to pile up, despite the well-founded objections of biochemists and immunologists. At the same time, the valid objection, that non-specific proteases can be demonstrated in tissue- and blood-cells and in serum, is equally factual. Oppenheimer points out that the serum enzymes are chiefly "tryptases" and "peptidases." A. Schmitz gave clear indication of a complex plasma system composed of (1) inactive precursor or zymogen (for which we should like to suggest the term "tryptogen," in analogy with pancreatic trypsinogens); (2) specific antitryptase; (3) kinase (activator) required for the tryptase to be liberated in active form. The analogy to the pancreatic trypsin system (ref. Northrop) is complete.

As far back as 1913, Collingwood and MacMahon produced thrombinolysis and hence inhibition of coagulation of blood by trypsin and suggested possibilities for the theory of clotting. Eagle (1937) reviewed subsequent work on trypsin in relation to blood coagulation. When Northrop and Kunitz showed their crystalline trypsin to be a coagulant, and Eagle, followed by the author, both using the crystalline enzyme, confirmed earlier work showing that the mode of action was "thromboplastic," the way was opened for reinvestigation of the old data on blood enzymes. It had long been known that blood serum is able to inhibit preparations of pancreatic trypsin. Northrop's crystalline trypsin-inhibitor (from pancreas) prevented trypsin from clotting $MgSO_4$-plasma. The author, in some unpublished experiments[1] with a similar inhibitor preparation (supplied through the courtesy of Dr. T. E. Weichselbaum, of Washington University, St. Louis), finds evidence of inhibition of ordinary clotting (!) of recalcified plasma by the crystalline inhibitor. The data indicate anti-fibrinolytic as well as anti-prothrombic and anti-thrombic effects. A co-factor is necessary, at least for the last, and can be supplied in crude plasma albumin. These actions closely resemble *heparin,* but are much weaker. Can it be that the pure polypeptide has a "heparin-like" acid-prosthetic group? Heparin is anti-tryptic.[2]

Ferguson believes that the natural blood tryptase (when freed from inhibitor and "activated") is the "missing-link" in the "thromboplastic" activation of prothrombin to thrombin. If present in sufficient amount and under adequate conditions, this tryptase can also *digest:* (1) prothrombin and thrombin (hence constituting "progressive" antithrombin);

[1] Ferguson, *Proc. Soc. Exp. Biol. and Med.*, 1942 (in press).

[2] Horwitt, SCIENCE, 92: 89, 1940; Glazko and Ferguson, *Proc. Soc. Exp. Biol. and Med.*, 45: 43, 1940.

(2) fibrinogen and fibrin[3] (hence the term "fibrinolysin" is superfluous); (3) non-specific substrates such as casein (in which small increases in formol-titration and N.P.N. have been occasionally observed). There can be little doubt that "active" tryptase is not normally present in the blood but requires "damaging" or disturbing procedures such as trichloracetic acid precipitation, shaking with chloroform (long known as an "antitrypsin" neutralizer), dialysis, etc. Still to be explained are the exact mechanisms of such "damage" in shed blood, but the fact that profound disturbances in colloidal equilibria do accompany "wetting" (contact with foreign surfaces) and the whole set of conditions encountered *in vitro* can not be denied. Autolysis of damaged tissue cells is an analogy, and the ease of trypsinogen activation is confirmed by Northrop's experiments with the crystalline substance. That "tryptogen" activation proceeds *autocatalytically* is suggested by some recent experiments[4] relating tryptase liberation to actual clotting.

The wide applicability of the new theory is indicated by the following chosen illustrations:

(1) Hemophilic[5] plasma (*in vitro*) is readily clotted by trypsin (and crude tissue "thromboplastins") when cephalin, alone, is of little avail.

(2) Danger of intravascular coagulation at present offers an insuperable obstacle to intravenous use of trypsin as a hemostatic, but ways of overcoming this serious obstacle may possibly be found.

(3) Serum-tryptase accounts for the instability and spoilage of plasma and plasma protein preparations. At present these difficulties are being overcome, very satisfactorily, but empirically, in the war production of plasma substitutes and hemostatic agents.

(4) Many anomalies in the experimental literature on blood-clotting find a ready explanation in the light of the various actions of trypsin.

(5) Close links are forged between blood-coagulation and immunological fields, and the two fields merge in the consideration of "transfusion reactions" and anaphylactic shock.[6]

In summary, the new clotting theory revolves around the proteases of the blood (plasma and formed elements) and tissue cells. Normally, the enzyme is not in the active state. "Damage," *i.e.*, colloidal disturbance, introduces new conditions favorable to activation of the enzyme. Tryptase, optimally in conjunction with ionized calcium and free phospholipid (cephalin), acts as a "thromboplastic" agent for the conversion of prothrombin to thrombin.[7] Thrombin is enzyme-like in potency and in the kinetics of its interaction with fibrinogen to form fibrin. Proteolytic phenomena such as fibrinolysis and a trace of ordinary protein cleavage are variable and due, not to thrombin, but to continuing action of serum protease. We can leave to the immunologists the problems of "foreign" protein cleavage but suggest that they look for quantitative changes in normal protease-inhibitors.[6]

In this theory, the important natural anticoagulants are tryptase-inhibitors. They seem to be present in normal plasma (and serum) and may be polypeptides with acidic (heparin or heparin-like) prosthetic groups. We do not wish to go so far as to assert that they must have the chemistry of heparins (mucoitinpolysulfuric-esters, Jorpes *et al.*). Our erstwhile colleague, Dr. A. J. Glazko, now on active service with the U. S. Navy, clearly showed that heparin is "specific" only in a physico-chemical sense. Analogous inhibitions may be produced with many agents, *e.g.*, polyvalent anions, sulfonic dyes and certain lipids (Jobling and Peterson; Chargaff), also the *basic* protamines (Ferguson, *et al.*). The antithromboplastic, anti-prothrombic and anti-thrombic actions of these inhibitor mechanisms form three defense lines against the untoward possibility of coagulation *in vivo*.

Our hypothesis combines the Morawitz and Howell and Nolf view-points by bringing all three into line

[3] Timing of fibrinolysis with the aid of the Evelyn photoelectric colorimeter is recommended as a simple and very sensitive method for assay of active tryptase (Ferguson, *Proc. Soc. Exp. Biol. and Med.*, 1942, in press). Clot-retraction (syneresis) is merely incipient fibrinolysis (Hirose, 1934; Ferguson, 1939). Tagnon's recent fibrinolytic studies (*Jour. Lab. and Clin. Med.*, 27: 1119, 1942) afford strong support for the major premises of the Ferguson theory.

[4] Ferguson, *Jour. Lab. and Clin. Med.* (in press, 1942).

[5] Feissly (*Helvet. Med. Acta*, 8: 823, 1941) uses a gelatine liquefaction technic to confirm the suggestion of Ferguson (*Am. Jour. Physiol.*, 126: 669, 1939) that hemophilia is a plasma defect in available protease. This view is further confirmed by Tagnon, Taylor, *et al.*, *Jour. Clin. Invest.*, in press, 1942.

[6] Burdon, *Proc. Soc. Exp. Biol. and Med.*, 49: 24, 1942.

[7] The fact that we have prepared prothrombin incapable of activation by Ca alone (despite the presence of cephalin, bound to the protein, demonstrated by analysis) and that the further addition of the pure "free" phospholipid quickly and completely converts it into a stable thrombin able to produce stable fibrin clots (*i.e.*, no evidence of tryptase), causes us, tentatively, to hold on to the view that the thromboplastic role of tryptase consists in catalyzing the formation of a prothrombin + calcium + cephalin "intermediary" complex in thrombin formation. The "final" thrombin can be obtained completely free from Ca and P-lipid. The proved ability of tryptase to activate prothrombin in oxalated or citrated materials (in which Ca-ions, as well as Cephalin, are "bound") is somewhat difficult to fit in with our (1936–1939) "cephalin availability" theory and the answer must be found in the future preparation of a purified prothrombin, completely freed from all, even bound, calcium, phospholipid and serum-tryptase. It is to be regretted that several recent attempts at prothrombin purification have been so concerned with the question of potency as to give scant attention to the equally significant problem of getting rid of these known chemical impurities, to which important characteristics of the allegedly "pure" preparations may very well be due.

with the newer knowledge of tryptase enzymes and their inhibitors. The subject is necessarily complex in view of the difficult and imperfectly understood chemistry. Nevertheless, we believe our solution to be a logical one and sufficiently in accord with a wide knowledge of experimental fact to satisfy the requirements of a "working hypothesis." Such is valuable in the assessment of current progress, in planning experiments for the future, and, above all, in its clear applications to immediate practical aims.

OBITUARY

DR. SUSAN P. NICHOLS, 1873–1942

DR. SUSAN P. NICHOLS, emeritus professor of botany of Oberlin College, was born at Brownville, Maine, on May 12, 1873, and died at her home in Portland, Maine, on December 7, 1942. She was graduated from the Brunswick High School and Bradford Academy, and from Cornell University with the degree of B.S. in the class of 1898, remaining at Cornell as a fellow, 1898–99. During the following year she occupied the American Woman's Table at the Naples Zoological Station. After teaching science at Houghton Seminary, New York, she studied at the University of Wisconsin, receiving her Ph.D. in 1904, with a thesis on Binucleate Cells in Basidiomycetes.

Following two years of teaching in a private school in Kentucky, she came to Oberlin as an instructor in botany in 1908 and remained at that institution until the end of her teaching career. In 1925 she was made professor, and on the retirement of Professor Frederick O. Grover, succeeded him as head of the department. This position she held until her own retirement in 1938. Her membership in scientific organizations included the American Association for the Advancement of Science, American Society of Naturalists, Botanical Society of America, Ohio Academy of Science and the Josselyn Botanical Society of Maine.

Her published work, other than the thesis mentioned, has to do with the physiology of algal cells, but her final illness interrupted work on native orchids in which she had been engaged for some time. This brief record does insufficient justice to her services for science. Her influence on students was notable. Despite the handicap of a withered arm, she was a skilled technician who aroused in her students an enthusiasm for clean and careful technique. She was blessed with a forthright and lucid New England mind which could give direction and perspective to this skill. The impact of her rugged honesty on the minds of her students was powerful and salutary. This honesty, with her common sense and force of character, also won for her a position of influence in the councils of the democratically governed college on whose faculty she served for thirty years. PAUL B. SEARS

DEATHS AND MEMORIALS

DR. LEONHARD STEJNEGER, head curator in biology at the U. S. National Museum, died on February 28 in his ninety-second year.

DR. LILLIEN JANE MARTIN, professor emeritus of psychology at Stanford University, died on March 26 at the age of ninety-one years.

DR. WILLIAM EDGAR CALDWELL, professor of clinical obstetrics and gynecology at the College of Physicians and Surgeons, Columbia University, and associate director of the Sloane Hospital of Columbia University, died on April 1. He was sixty-three years old.

JOHN A. NEWLIN, who retired from the staff of the U. S. Forest Products Laboratory at Madison, Wis., on March 1, died on March 27 at the age of seventy-one years. He had been with the Forest Service since 1904.

PALEMON HOWARD DORSETT, agricultural explorer, recipient of the Meyer Medal for distinguished service, for forty-one years horticulturist with the U. S. Department of Agriculture, died in Washington, D. C., on April 1 at the age of eighty-one years.

MAJOR LEONARD DARWIN, fourth and last surviving son of Charles Darwin, died on March 21 at the age of ninety-three years.

PROFESSOR ANTONIO CARDOSO FONTE, Rio de Janeiro, Brazil, formerly director of the Instituto Oswaldo Cruz in Rio de Janeiro, known for his work on tropical diseases, died on March 27 at the age of sixty-three years.

A RESOLUTION to declare February 11, 1944, Thomas Alva Edison Day in memory of the inventor, was approved on March 29 by the Judiciary Committee of the U. S. Senate.

A MONUMENT to the memory of William H. Jackson, pioneer artist and photographer of the early West, was dedicated on Sunday, April 4, at Arlington National Cemetery by the Explorers' Club of America and the American Pioneer Trails Association. The occasion was the one hundredth birthday of Mr. Jackson, who died last year at the age of ninety-nine years. Mr. Jackson made the first photographic record of "Old Faithful" in Yellowstone National Park. He later served as official photographer with the U. S. Geological Survey.