Symposien — Conferences — Conférences

Eighth Annual Symposium on Blood

January 16 and 17, 1959*)

McGregor Memorial Conference Center

Committee:

WALTER H. SEEGERS, ELWOOD A. SHARP, J. FREDERIC JOHNSON

Procurement, Processing and Administration of Human Bone Marrow for Attempts at Transplantation. Repplinger, E., Haurani, F., Christofanini, A., Long, E. R. and Tocantins, L. M., Charlotte Drake Cardeza Foundation, Jefferson Medical College, Philadelphia, Pennsylvania.

During the past few years much work has been done in bone marrow transplantation in animals. Earlier it was shown that animals can be protected against total body irradiation by injections of suitable marrow material. Subsequently it was found that murine leukemia could be controlled when the leukemic animals received total body irradiation followed by infusion of homologous marrow.

These experimental results in animals stimulated us to try and influence the course of acute leukemia and aplastic anemia in man. Bone marrow suspensions were prepared from excised ribs of healthy donors. A total of 2 to 18 billion marrow cells have been infused at one time without any immediate or remote reactions. Patients with aplastic anemia received no radiation, while those with acute leukemia received total body irradiation from 40—600 r. Both groups received steroids and other supportive therapy. One patient with acute leukemia received large doses of antimetabolites without X-ray treatment.

On the whole, the results were poor and there was no evidence of "take" of the infused bone marrow. However, there were some temporary improvements and, in one patient, there was a remission of seven months. The complications of total body irradiation as such, demand a great deal of attention and renders this mode of therapy a difficult one to handle.

Some Bleeding Disorders Related to Platelet Factor 3. Johnson, S. A., Monto, R. W. and Rebuck, J. W., Henry Ford Hospital, Detroit, Michigan.

A basic pattern in coagulation has been observed in bleeding disorders where platelet factor 3 is involved. In the thrombocytopenic state, 50 000 mm^3 platelets or less, a poor prothrom-

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bin consumption time is observed in the absence of residual prothrombin in the serum. The short prothrombin consumption time is due to the formation, perhaps from prothrombin, of a platelet prothrombin-like factor, which is certainly similar chemically to prothrombin. This same pattern is present when platelet factor 3 is unavailable for blood clotting in congenital thrombocytopathy and the acquired thrombocytopathy of uremia, even though platelet numbers are normal. The defect in platelet factor 3 in each of these bleeding problems is vastly different both physiologically and morphologically in the face of this similar pattern. The authors believe this pattern is characteristic of all platelet factor 3 abnormalities.

Experimental Studies with 5-Hydroxytryptamine (Serotonin) as a Hemostyptic Agent Following Traumatic Injury After Irradiation in White Rats. Brambel, Ch. E. and Murphy, D., University of Notre Dame, Notre Dame, Indiana and Northwestern University Medical School, Chicago, Illinois.

A decrease in the volume of blood lost as a consequence of traumatic injury during experimentally induced thrombocytopenic states (irradiation and antiplatelet serum) has been observed following intraperitoneal injections of 5-hydroxytryptamine. The procedure described by the authors (Circulation Research 6: 456 [1958]) was used to study blood loss in rats following lethal total body irradiation and in non-irradiated control rats.

The non-irratiated control rats were bled a mean of 4.0 ml. Decreased bleeding was observed in the irradiated rats during the first four days after exposure to the effects of ionizing rays. The radiation injured animals bled one-half as much as the corresponding non-irradiated controls. The classical bleeding syndrome associated with radiation sickness was observed on the 7th, 8th and 9th days post-irradiation, during which time the radiation injured rats lost a greater volume of blood when compared to non-irradiated control animals. This enhanced bleeding coincided with the drastic depletion of blood platelets in the peripheral blood.

5-HT in a dosage of 3 mg/kg was found to be highly effective in controlling bleeding from traumatic injury following total body irradiation. For the first five days after irradiation, the response elicited by this compound in the irradiated rat was indistinguishable from the corresponding effect in the control animal, i.e., 0.1 ml blood. It may be of some interest to note that a diminished response to 5-HT was observed during the time of maximum thrombocytopenia suggesting that circulating blood platelets are essential in this interaction. Other amines (epinephrine and norepinephrine) associated with platelets did not decrease blood volume loss.

A decrease in mortality rate from hemorrhage associated with traumatic injury in post-irradiation states was observed after parenteral administration of 5-HT. All of the irradiated rats died as the result of the bleeding procedure which were bled on the days of maximum platelet deletion that did not receive 5-HT.

Purification and Identification of Phospholipides with Procoagulant Activity from Brain, Platelets and Soybean. Jensen, H., Therriault, D. G. and Nichols, Th., US Army Medical Research Laboratory, Fort Knox, Kentucky.

The term *procoagulant* is used for a phospholipide preparation which on interaction with certain clotting factors yields a prothrombin activator.

Utilizing fractional precipitation with organic solvents, countercurrent distribution and column chromatography, phosphatidyl serine and lecithin were obtained in chromatographically pure form from beef brain tissue. Measurements of the procoagulant activities of these phosphatides by determining their influence on the recalcification time and on the formation of thrombokinase in the thromboplastin generation test revealed that phosphatidyl serine or lecithin alone had only little or no procoagulant activity. However, when these phosphatides were allowed to react together in a chloroform solution, the reaction product, obtained after evaporation of the chloroform, was found to exert a very pronounced procoagulant activity.

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Employing methods similar to those used in the study on brain tissue, comparatively pure preparations of the following phospholipides were obtained from beef platelets: Phosphatidyl ethanolamine, phosphatidyl serine, lecithin and sphingomyelin. The phosphatidyl serine content of bovine platelets seems to be relatively small. None of the four phospholipide preparations exhibited any appreciable procoagulant activity when tested alone. However, mixtures of phosphatidyl serine with either lecithin or sphingomyelin or of phosphatidyl ethanolamine with either lecithin or sphingomyelin showed pronounced procoagulant activity.

Apparently the procoagulant activity of soybean phosphatide fractions can be attributed mainly to phosphatidyl ethanolamine, since thus far no evidence for the presence of phosphatidyl serine has been obtained. Again it was found that the phosphatidyl ethanolamine alone showed no procoagulant activity, but following mixing with lecithin, procoagulant activity was generated.

These findings support the supposition that the presence of certain "procoagulant-active" phospholipides in the diet may conceivably contribute to hypercoagulability of blood and consequently to the development of atherosclerosis and possible coronary occlusion.

Properties of Anti-Plasmathromboplastin. Deutsch, E. and Mammen, E., University of Vienna, Vienna, Austria.

In continuation of former studies by E. Deutsch and H. Fuchs (VI Conf. Europ. Soc. Haemat., Copenhagen, 1957) we investigated the physical and chemical properties and the working mechanism of the anti-plasmathromboplastin. We found in the normal human serum two substances with anti-thromboplastic properties. Both migrate in the electrophoresis with the alpha-globulin fractions. We were able to separate these substances by different fractionations, such as ammonium sulfate fractionation, ethanol fractionation and changing the pH. They differ from each other in their heating stability, their storage stability, their adsorption on cation exchange resins, their dialysis properties, their curve of inactivation, and in their effect on dilution. Both need no calcium in the incubation mixture and their action seems to be stoichiometric.

Intracellular Distribution of Prothrombin Regenerating Capacity. Barnhart, M. I. and Hollatz, R. G., Wayne State University College of Medicine, Detroit, Michigan.

That mitochondrial preparations have the ability to regenerate prothrombin activity from several different "inactive" prothrombin derivatives has been established. The major objective of the present study was to discover if the observed regenerating activity was limited to mitochondria or was distributed among other cellular fractions as well.

Cellular fractions of rat liver were prepared following the techniques of Schneider and Hageboom and adjusted to a 10% solution with 0.25 M sucrose. Sub-fractions of mitochondria were obtained according to de Duve and associates. The substrate for the regeneration system was an "inactive" prothrombin derivative prepared with the use of liver mitochondria. For each cellular fraction conditions for maximal regeneration of prothrombin activity were determined. The regenerative capacity was not lost during prolonged freezing. Thrombin could not be detected as a by-product in either the inactivation or regeneration phase.

Measurements of specific activity (units of regenerated prothrombin/min./mg protein) for each cell fraction were made to determine the intracellular distribution of the capacity to regenerate prothrombin at pH 7.8. This ability was concentrated within microsomes and light mitochondria. Microsomes were found to have four to five times and light mitochondria two to three times the capacity of either the total homogenate or cytoplasmic extract.

Enhancement or inhibition of the regeneration reaction occurred when the cellular fractions were recombined in various ways. Augmentation of individual yields occurred when particulate fractions were combined in sub-optimal concentrations. However, the supernatant (soluble fraction) inhibited the regenerating capacity of the particulate fractions singly or combined.

Studies were also made with cellular fractions from the kidney, spleen and heart of the rat, as well as from organs of the rabbit. Cellular fractions from the liver and kidney were most active in the regeneration of prothrombin, but fractions from all organs exhibited some ability. Concentration of this capacity in the microsomes was clearly demonstrated.

The Psychology of Science. Dorsey, J. M., Wayne State University College of Medicine, Detroit, Michigan.

"There are more false facts current in the world than false theories."
Cullen

"You can have neither a greater nor a less dominion than that over yourself."
Leonardo da Vinci

By "psychology" I mean: my study of my mind's existence itself. By "science" I mean: my mind's self-activity systematically directed towards developing my potential manpower.

Every "system of scientific procedure" is an outgrowth of a "system of psychlogy", quite as every system of psychology is an outgrowth of life's "human system." An educator who appreciates the basic fact that his psychological orientation decides his educational orientation, finds it necessary to do some research upon the order of his mind. Regularly he discovers that he has obscured, almost to the vanishing point, these mind strengthening truths: 1) all of his "facts of life" are nothing but evidence of the *fact of his life*, 2) all of his scientific data are nothing but his own self data, 3) all of his research (learning) experience is nothing but his own self-activity furthering his own self-development, 4) all of his refinement of himself as a scientific instrument (means), has been at the expense of his appreciation of himself as an *end*, 5) all that *can* reveal his life as worth living, *is* the realization that it is his own, 6) all that *can* support disesteem for his life is his (piling up) disregard for his experience as entirely self-experience, 7) all of the health benefit of his scientific effort is safeguarded, if he steadily heeds it as augmenting his (ever increasing) estimate of his own worth, 8) all humanization of science (the countervailing force now needed to prevent scientific suicide) can derive only from the scientist's cultivating the selfinsight enabling him to call his scientific soul his own, 9) all educational programs are decided ultimately by what the educator has been able to achieve of the insight: My mind consciousness decides the limits of my educational perspectives, 10) all that *can* be of any importance to anyone is himself, hence the advantage of growing to see one's world as one's own.

All knowledge obtained by careful inquiry study and other experience, all systematized knowledge, all observation and classification of facts, all critical exhaustive investigation and experimentation, all principles and practices of exact scientific research, — as well as whatever else is lived in the name of Science, needs must be lived in the name of the given scientist, in order for that scientist's healthy mental development to obtain.

I find psychotherapy (the process of appreciated selt-discovery essential for wholesome self-esteem) to be the specific antidote for scientific training (and for all formal and informal education) based upon disregard for the inviolability of human individuality.

The urgent and constant health need of the scientist is to identify correctly all of his findings as "the business of living" his personal life. Laboratory work, experimental projects, scientific discoveries, — all are wholesomely lived only as recognized and *appreciated* developments of *his* scientifically disciplined life. The scientist's discovery of the vastness of his world must spell out for him his own littleness, each of his scientific developments must dwarf his own recognition of his magnanimity, unless he develops the habit of mind of noticing that all of his life's experience (including his scientific research) consists of his self-knowledge and thus enhances his own worth.

Systematically observing myself as a fount of creative power, methodically studying my human being as a growing life which is everyday in the making, crediting myself with

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the realization that I have a truly marvelous mind which works scientific wonders for me, — all such getting to work of this special part of my mind, this highest helpfulness which I call "self-consciousness", is a life-saving kind of accounting. It is this sense of selfness which puts value on life. And my possession of my life is my one and only true possession, my any other claim of property being a true illusion. "Anti" self-culture scientist, is contradiction in terms.

As Ruskin recorded, "there is no wealth but life". My only discovery worthy of the name is a growth of my mind, a new mental development. I can be sure of my ground really if I can recognize it as mind, otherwise not. My scientific research prospers when I can put to work the certainty of my conscious oneness of *my* world.

It is all which I cannot see as going on *within me,* which suffers me to live any of my scientific work as if it were not personally human. It is my scientific discipline which I can see as my inner workings, which commits me to a kind peaceful search for the attainment of human well-being, to a careful and caring observance of every law that concerns the furtherance of life.

My whole wisdom of life consists in my proper appreciation of LIFE as being that power which alone can make anything possible for me. What gives my presence of mind, my momentary self-awareness, an estimate of genuine solid worth, is my regard for myself as a growing life, as a developing human being, as a treasure of my self's experience. What every scientific meaning is in terms of *my own mind's creating it,* — therein is its only truth (reality, scientific validity) to be found. The highest reach of human science is the clear scientific recognition of the scientist's psychological world. Otherwise must obtain the definition of the scientist as: one who knows more and more (of illusional externality) about less and less (of his own human development). "Learned ignorance", the consciousness of his ever expanding room for his further human development, is the self-conscious scientist's reward for "hanging on" to his scientific self, for feeling equal to himself.

> "Read not to contradict and confute, nor to believe and take for granted — but to weigh and consider."
>
> Francis Bacon, 1620, Novum Organum

> "Man one harmonious soul of many a soul Whose nature is its own divine control."
>
> Shelley

Studies of the Effect to Thorotrast on Blood Coagulation. Bodi, T., Holburn, R. R., Geldner, J., Carroll, R. T. and Tocantins, L. M., Charlotte Drake Cardeza Foundation, Jefferson Medical College, Philadelphia, Pennsylvania.

Prolongation of the venous blood clotting time after the injection of Thorotrast (thorium dioxide in 25% dextrin) with 3.8% Sodium Citrate has been observed in patients receiving the drug intraarterially for cerebral angiography. A similar effect was observed in rabbits and dogs. The prolongation of the clotting time of whole blood and recalcified plasma appeared 15 minutes after injection and persisted several hours. The platelet count of the patient was unaffected but the plasma prothrombin time was increased. Thromboplastin generation was also markedly decreased. Addition of protamine to the whole blood or plasma *in vitro* corrected the defect in clotting times. Allowing the blood or plasma to stand several hours before testing resulted in the gradual loss of hypocoagulability and ended in hypercoagulability. The *in vivo* studies suggest the release of an endogenous heparinoid substance as the cause of the coagulation defect. The injection of citrate alone into dogs did not produce a change in coagulation. *In vitro* the addition of Thorotrast in high concentrations to whole blood resulted in lengthened clotting times but was ineffective when added to plasma.

Observations on Intravascular Clots. Murray, M., University of Wisconsin School of Medicine, Madison, Wisconsin.

Laki and Lorand (Nature 166: 694 [1950]; Science 108: 280 [1948]) observed that clots formed by the recalcification of plasma were insoluble in 5 M urea, whereas clots formed by the conversion of purified fibrinogen with purified thrombin were soluble in 5 M urea. In evaluating clots formed by the action of purified Staphylococcus coagulase on purified fibrinogen or plasma, it was found that such clots were soluble in 5 M urea. In view of the fact that clots from shed blood were insoluble it seems desirable to study the denaturation characteristics of fibrin clots from autopsy material.

Post mortem clots (chicken fat and red jelly clots) were uniformly soluble in the urea solutions. Evaluation of eleven cases of ante mortem thrombosis in which three mural thrombi of the heart, one of the vena cava, one of the renal artery and six from the pulmonary veins were all partially soluble in 5 M urea. The degree of solubility varied with the age of the clots and consequent degree of organization. Clots less than four days of age varied in solubility from 80 to 90%.

Histologic observation of residues of clots after incubation in urea revealed cellular elements and small amounts of fibrin. One clot from a case of a ruptured liver following needle biopsy was evaluated for its solubility in 5 M urea. This clot was not soluble in urea. In view of the fact that fibrin clots formed in the vessels and those formed outside the vessels have different chemical properties, i. e. degree of solubility in 5 M urea, it is reasonable to assume that they may have been formed by different mechanisms.

The Non-Enzymatic Induction of Fibrinolysis. Weiner, M., Redisch, W. and Weisberg, L., New York University Research Service, Goldwater Memorial Hospital New York, New York.

The in vivo induction of mild degrees of fibrinolysis by non-enzymatic substances has been reported for several compounds with vasomotor activity. The intravenous administration of nicotinic acid in man has recently been found to result in fibrinolysis. (Proc. Soc. Exper. Biol. 98: 755 [1958]). The phenomenon was not found to occur in dog, rabbit, rat, or guinea pig, nor does it result from the in vitro addition of nicotinic acid to human plasma. In spite of clear evidence of absorption in man, oral nicotinic acid fails to induce fibrinolysis. Only in one patient, a cirrhotic with a portocaval shunt, did oral nicotinic acid induce lysis, suggesting that initial circulation of all the administered nicotinic acid through the liver is incompatible with fibrinolysis activation. After single doses of nicotinic acid, oral or parenteral, the subject will not respond with lysis to subsequent parenteral doses for several hours or days. Continuous oral medication is followed by prolonged periods (weeks or months) of resistance to the usual lysis pattern of subsequently administered intravenous nicotinic acid.

Other flush producing and vasomotor drugs studied all failed to cause the clear fibrinolysis seen by "thrombelastograph" and test tube observation of plasma specimens after parenteral nicotinic acid. Nicotinamide also does not induce lysis, but does alter the usual lytic response of subsequent parenteral doses of nicotinic acid.

Removal of the clot of a post-nicotinic acid lytic specimen from its serum does not prevent its lysis, presumably because of adsorbed fibrinolysin. Normal clots added to the residual serum are less likely to dissolve than the native clot, although lysis of the normal clot has been clearly observed in some instances. Problems related to clinical application as well as theoretical explanation of the observed phenomena will be discussed.

Urokinase Studies. von Kaulla, K. N., University of Colorado School of Medicine and Belle Bonfils Memorial Blood Bank, Denver, Colorado.

Human urokinase is readily prepared by adsorption on porcelain. Pooled urine is filtered through a Coors filtering cylinder (porosity one). It is washed back first with water, eluting urine "thromboplastin", and then with 1/20 volume 1 m KSCN, eluting urokinase. The KSCN-solution is cleared by centrifugation, concentrated to 1/10 by evaporation with simultaneous dialysis, and finally lyophilized after further dialysis against dist. water. The yield of urokinase is 12—24 units (4—6 mg) per 1000 ml urine. 1 ml of pooled urine contains 0.025—0.05 units

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of urokinase. In this laboratory, one unit of human urokinase is defined as the amount, added before clotting, which is required to induce dissolution of one ml of thrombin-clotted human ACD-plasma within 30 min. at 37° C. Human urokinase incubated with human citrated plasma produces a progressive prolongation of the thrombin time, this is instantly normalized upon the addition of $CaCl_2$. Applied on human plasma plates, the urokinase makes it possible to test the effect of various substances on a homologous human fibrinolytic system. Thymol blue (20 µg/ml), heparin (5 µg/ml) and a protein free urine fraction consistently enhance fibrinolysis in this system. Urokinase-induced digestion areas on human plasma plates grow very rapidly for few hours and then level off. Human urokinase excretion, measured quantitatively (von Kaulla, K. N., Schneeberger, E., Curry, M.: Fed. Proc. 17: 167 [1958]) in connection with cardiac surgery, reveal a decrease after the operation to a fraction of the preoperative values. The minimum excretion occurs on the 3rd—4th postoperative day; then, there is subsequent gradual return to preoperative values. In both surgical and medical patients, a sudden rise of urokinase excretion to a considerably greater level than precedent values can be an indication of imminent bleeding. These changes in the excretion rate are independent from creatinine excretion or specific gravity of the urine. Our previous studies indicated that some types of surgery and pyrogen-induced fibrinolysis in man can also increase urokinase excretion.

In the search for more satisfactory stimuli to induce fibrinolysis and the alteration of urokinase excretion in man, it was found that i.v. $CaCl_2$, Ronicol Compositum, and other drugs bring about a rather pronounced fibrinolytic reaction within minutes, depending on the injection speed. This finding warrants further extensive search for synthetic compounds inducing therapeutically useful fibrinolysis.

Purification of Urokinase and Some Characteristics of the Product. Celander, D. R., Schlagenhauf, Jr., G. K. and Guest, M. M., University of Texas — Medical Branch, Galveston, Texas.

Human urokinase has been brought to a high degree of purity by procedures involving its approximately 100-fold concentration by the foam technic (Celander, Langlinais, and Guest, Arch. Biochem. Biophys. 55: 286 [1955]), further purification by Chromatography on silicic acid (Ploug and Kjeldgaard, Biochem. Biophys. Acta 24: 278 [1957]) followed by ammonium sulfate fractionation and separation from remaining impurities by means of chromatography on diethylaminoethyl (DEAE)-cellulose. The procedure is quantitative through the ammonium sulfate fractionation; the success of the second chromatographic step depends upon obtaining a satisfactory lot of DEAE-cellulose. The material so prepared has approximately 6000 units urokinase (Celander, *et al., loc. cit.*) per milligram protein, corresponding to approximately 60,000 units per milligram protein when assayed by the method of Ploug and Kjeldgaard *(loc. cit.)*. From the data of the latter authors, the preparation appears to be nearly homogeneous. Under optimal conditions the overall yield approaches 100 per cent. Urokinase so derived has been used (1) in the study of profibrinolysin contamination in human fibrinogen prepared by the freeze thaw technic with the resulting data indicating that about 0.03 per cent of the profibrinolysin present in human plasma is retained in the average freeze-thaw preparation of human fibrinogen; (2) in pyrogenic studies in which 0.36 mg of the material, injected into the marginal ear vein of rabbits, produced a 1—2° C temperature rise; and (3) in studies of antigenicity in guinea pigs and rabbits. In guinea pigs a total of 0.18 mg urokinase injected in divided doses over a period of one month produced a degree of sensitization sufficient to cause acute anaphylaxis and death when the animal was challenged with 0.18 mg of the same preparation or with urokinase prepared locally by the method of Ploug and Kjeldgaard. Urokinase, isolated from the urine of rabbits injected with human urokinase, produced similar anaphylaxis in sensitized guinea pigs. Although neither anaphylaxis nor a precipitation reaction was obtained with urokinase in presumably urokinase sensitized rabbits, the serum globulin of these animals was more inhibitory to urokinase than was the globulin of normal animals. The significance of these findings is discussed.

Epsilon Aminocaproic Acid: an Inhibitor of Plasminogen Activation. Alkjaersig, N., Fletcher, A. P. and Sherry, S., Washington University School of Medicine, St. Louis, Missouri.

Numerous substances have been described as plasmin inhibitors, but only in a few cases, due to the ambiguity of the methods used, has distinction been drawn between inhibition of plasminogen activation and inhibition of plasmin action. ε-aminocaproic acid, a newly described "plasmininhibitor" (British Patent Office Specification No. 770, 693 [1957]) has been investigated not only on account of the intrinsic interest attached to the compound itself, but also as a model substance suited to the development of adequate methodology for such studies. Systems were developed for the obtaining of kinetic data and testing was performed against the plasminogen activators, streptokinase, urokinase and fibrinokinase. The effect of ε-aminocaproic acid was also tested on the enzymes, trypsin and plasmin, which possess both proteolytic activity and plasminogen activator activity (plasmin acts by autocatalytic action). ε-aminocaproic acid competitively inhibited the activation of plasminogen by streptokinase, urokinase and probably fibrinokinase, but inhibited plasminogen activation by trypsin non-competitively. The results were similar whether the appropriate test system contained human plasminogen or bovine plasminogen. ε-aminocaproic acid in concentrations exceeding 0.06 M was a non-competitive inhibitor of the proteolytic activities shown by plasmin or trypsin, but in lower concentrations it enhanced the proteolytic action of plasmin. The results supported the concept that plasminogen activation may occur by two mechanisms yielding plasmins with similar biochemical activities, but of different molecular size (J. Biol. Chem. **233:** 86 [1958]). The inhibitory actions of ε-aminocaproic acid on plasminogen activation were demonstrable at much lower concentrations than were required for plasmin inhibition. Where, as in plasma, both activities may occur together, the ability of ε-aminocaproic acid to inhibit plasminogen activator at concentration insufficient to effect plasmin activity permits their separate effects to be distinguished.

Thrombolytic Therapy in Patients Suffering from Myocardial Infarction and other Thrombo-embolic States. A Consideration of its Production Effects and Hazards. Fletcher, A. P., Sherry, S. and Alkjaersig, N., Washington University School of Medicine, St. Louis, Missouri.

Thrombolysis, a term used to designate the lysis of thrombi or emboli, is mediated by a mechanism involving the absorbtion or diffusion of plasminogen activator to a thrombus, consequent activation of intrinsic thrombus plasminogen and resultant thrombolysis. The intravenous injection of a priming dose of streptokinase followed by the infusion of a suitable maintenance dose will produce, in man, a constant and high level of circulating plasminogen activator. Although plasma plasminogen and plasmin concentrations fall to undetectable levels, the plasma, by virtue of its plasminogen activator content, will lyse preformed isotopically labelled human plasma clots. The thrombolytic activity of the plasma measured under these test conditions, which approximate the experimental conditions in vivo, is of the order of 100—500 micrograms fibrin lysed/ml plasma/hour. Thrombolytic states of 30 or more hours duration have been induced, by means of this method, in 50 patients suffering from thromboembolic disease of whom 24 had early acute myocardial infarction. The induced thrombolytic state resulted in the production of a coagulation defect evidenced by a rise in the one stage prothrombin time due partly to a fall in plasma accelerator globulin content but more importantly to the development of plasma antithrombin activity. This activity was thought to be caused by the presence of fibrinogen breakdown products and its appearance could in part be prevented by the administration of hydrocortisone. Clinically there was evidence that in certain instances in vivo thrombolysis occurred as a consequence of the treatment and that therapy could be administered without deleterious consequence. Despite possible theoretical objections to its use in patients with myocardial infarction, 18 of 19 patients treated early survived for 6 or more months. It is emphasized that many problems remain for future solution.

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Calcium Phosphate Chromatography of Partially Purified Bovine Profibrinolysin (Plasminogen) and Antifibrinolysin (Antiplasmin). Dalby, A., Cole, E. R. and Mertz, E. T., Purdue University, Lafayette, Indiana.

A 3-fold purification of non-dialyzed bovine plasminogen (Amer. J. Physiol. **191**: 505 [1957]) was obtained by 40-fold dilution and adjustment of the pH to 5.3, the plasminogen being in the precipitate. A further 5- to 6-fold purification was obtained on a small scale by chromatography on columns of calcium phosphate gel (Arch. Biochem. Biophys. **65**: 132 [1956]). At pH 6.8 plasminogen is eluted in the sodium phosphate concentration range 0.05 M—0.10 M. This material appears to contain at least one major impurity and has a specific activity approximately equivalent to that of human plasminogen prepared by the method of Kline (J. Biol. Chem. **204**: 949 [1953]). After removal of plasminogen from bovine serum, antiplasmin was precipitated between 40% and 60% saturation with ammonium sulfate. The antiplasmin fraction was further purified by dialysis and precipitation at pH 5.0, the precipitate being discarded. The supernate had a specific activity about five times greater than the original serum. On small calcium phosphate columns, a further 10-fold increase in activity was obtained by elution of the supernate in the phosphate concentration range 0.003 M to 0.005 M at pH 6.8. The eluted antiplasmin was very unstable, and lost its activity in 3 hours at 5° C. Larger quantities of a more stable preparation, having a lower activity (7-fold increase only) but higher yield, were prepared by continuously applying the antiplasmin supernate in 0.04 M sodium phosphate to a column.

Prevention and Production of Thrombosis by Alterations in Electric Environment. Schwartz, S. I., University of Rochester School of Medicine and Dentistry, Rochester, New York.

This study represents an investigation into the association between bio-electric alterations and intravascular thrombosis. An electrically positive environment, achieved by imposing a direct current in the range of one to four milliamperes across a segment of vein which had been occluded proximally, consistently resulted in the formation of an intravascular thrombus. Heparin uniformally protected against this electrically induced thrombus, while dicumarol had no effect. The heparin protection was to be expected since injection of heparin markedly increased the electro-negativity of flowing blood in respect to the adventitia of the blood vessel, concomitant with its increase of clotting time. The imposition of an electrically negative field to a blood vessel prevented or markedly diminished the formation of a chemically induced intravascular thrombus. A current ranging between one and four milliamperes was used to make a segment of the femoral vein electrically negative. The opposite femoral vein was used as a currentless control and 500 units of topical thrombin were injected to both femoral veins which had been occluded proximally. After thirty minutes the veins were opened. In all instances a large thrombus had formed in the control vein while half the veins which were protected with a negative current contained no thrombi. The remainder had minimal thrombi which was significantly smaller than their currentless control. The summation of these results suggests that an electric alteration is one of the common denominators for intravascular thromboses, which occur subsequent to a variety of blood vessel trauma and change in the constituents of blood.

Observations on the Biosynthesis of Staphylocoagulase. Halick, P., Wayne State University College of Medicine, Detroit, Michigan.

The following relates to both the unique position staphylocoagulase occupies in the clotting of blood and to the close tie it bears the pathogenicity of *Staphylococcus aureus*.

This work was initiated in 1950 with the study of the relationship of coagulase to the blood clotting scheme as the objective. The development and use of a special growth medium made convenient for study a potent concentrate of coagulase. The medium, a dialysate of

heart infusion broth, was separated from the coagulase by dialysis and the coagulase was concentrated by lyophilization. Coagulase could also be removed by Seitz filtration. These coagulase preparations had no effect on conversion of fibrinogen in the presence of prothrombin activators. These and other observations offered no definition for the mechanism of coagulase clotting.

A number of special media were tested for coagulase production capacity. Often, luxurious growth coexisted with suppression of coagulase production. The possibility that these media presented varying amounts of material(s) necessary for coagulase synthesis was investigated. Dr. I. I. Szeto and I gave characterization to the coagulase production capacity of the dialysate of heart infusion broth. In all tested cases the substance(s) behaved as could a peptide.

The Amino Acid Composition of Thrombin Preparations. Miller, K. D., Brown, R. K., Casillas, G. and Seegers, W. H., Division of Laboratories and Research, New York State Department of Health, New York, New York, and Wayne State University College of Medicine, Detroit, Michigan.

Five bovine prothrombin preparations containing traces of accelerator globulin (Record Chem. Prog. **13:** 143 [1952]) were completely activated at optimum calcium concentration in the presence of an excess of sedimentable lung thromboplastin. After removing the thromboplastin by centrifuging, the thrombin was precipitated from 50 per cent cold acetone. Subsequently, the thrombin was separated by chromatography on the ion exchanger, IRC-50 (XE-64) as described by Rasmussen (Biochim. et Biophys. Acta **14:** 567 [1954]). The active protein was obtained from the chromatographic solvent, 0.3 *M* phosphate buffer, pH 8.0, by the addition of 3 volumes of saturated ammonium sulfate in the cold. The precipitate was collected by centrifuging and reprecipitated three times with 50 per cent acetone after dissolving each precipitate in water. These final thrombin products were dried to constant weight *in vacuo* at 58° C. The nitrogen content was 16.3 per cent of the dry weight. Constant boiling HCl which had been twice distilled in an all glass system was added in the proportion of 1 ml per 5 mg of thrombin protein. The solutions were sealed under vacuum and digested at 110° C for varying periods of from 17 to 72 hours. Very little humin formation was demonstrable. The hydrolyzates were dried *in vacuo*, the removal of HCl being insured by drying two subsequent aqueous solutions of the hydrolyzed materials *in vacuo*.

Amino acid composition of the hydrolyzates was determined by chromatography on Dowex 50-X4 by the procedure of Moore and Stein (J. Biol. Chem. **211:** 893 [1954]) employing an automatic, continuous-recording apparatus designed after Spackman, Moore and Stein (Analyt. Chem. **30:** 1190 [1958]). Tryptophane and tyrosine were determined by the spectrophotometric method of Holiday and Ogston (Biochem. J. **32:** 466 [1938]). No reducing sugar was detected on reacting the thrombin with anthrone in concentrated sulfuric acid. Corrections for loss of amino acids during hydrolysis are based on their loss from bovine pancreatic ribonuclease A hydrolyzed under identical conditions and include 7.5 per cent for serine, 4 per cent for threonine, 3 per cent for glutamic acid and 1.5 per cent for aspartic acid. The analytical results appearing in the table are from duplicate determinations of a single preparation but are in good agreement with the values for the other preparations analyzed.

The discrepancy between weight and nitrogen recovery remains a problem in considering this data. Sufficient quantities of thrombin were not available for ash determination. It is possible that the thrombin was prepared as the ammonium salt by the fractionation procedures outlined, thus yielding high ammonia figures. Prothrombin (Arch. Biochem. **49:** 276 [1954]) is relatively lower in lysine and higher in serine and alanine content than thrombin. The reducing sugar of the prothrombin apparently is a constituent of the non-thrombin fragment produced during prothrombin activation (Fed. Proc. **17:** 276 [1958]).

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The Amino Acid Composition of Bovine Thrombin

Residue	μM aa / 100 μM N	g aa residues / 100 g protein
ASP	6.05	8.10
THR	2.99	3.52
SER	3.48	3.51
GLU	7.06	10.86
PRO	3.44	3.89
GLY	5.44	3.62
ALA	3.04	2.51
CYS	1.85	2.20
VAL	3.95	4.55
MET	1.06	1.61
ILEU	2.61	3.45
LEU	5.85	7.70
TYR	2.36	4.83
PHE	2.66	4.22
GAL.NH_2(?)	0.64	1.08
LYS	5.32	7.93
HIS	1.37	2.18
NH_3	4.26	0.05
ARG	4.87	8.83
TRY*)	1.60	3.24
	94.17**)	87.88

*) Spectrophotometric determination — this determination gave 4.98 for TYR.

**) μM aa N recovered per 100 μM N

Metabolism of Isotopically Labeled Serum Albumin. Fleischer, S., Walter, H. and Haurowitz, F., Indiana University, Bloomington, Indiana.

Rabbits, guinea-pigs, rats and chicken were injected with amino acids containing S^{35} or C^{14}. A few hours or 1—2 days later the animals were bled; their plasma proteins were isolated and coupled with traces of I^{131}. If the doubly labeled serum albumin was injected into animals of the same or another species, we found invariably that the protein-bound radioactivity of the S^{35}- or C^{14}-amino acids persisted much longer in the circulation than the activity of I^{131}. We attribute the observed difference in the fate of the two isotopes to breakdown of some of the serum albumin in the tissues, excretion of iodinated breakdown products and reutilization of the internally labeled amino acids for the synthesis of new organ proteins (J. Biol. Chem. **224**: 107 [1957]).

Are the injected serum albumin molecules broken down randomly or are "old" molecules broken down first? To answer this question we injected one group of rats with serum albumin from rats killed 8 hours after the injection of S^{35}-amino acids and another group with the serum albumin of rats killed four days after injection of S^{35}-amino acids. We found no significant difference between the half-lives of the young and the old serum albumin molecules (Science **128**: 140 [1958]). This indicates that the serum albumin molecules are broken down randomly and not in dependence of their age. In other experiments (unpublished) we have injected one group of adult rabbits with the S^{35}-serum albumin of adult rabbits, another group with the serum albumin of new-born rabbits. The half-life of the serum albumins of both groups was approximately 6.5 to 7.0 days. In some of these experiments the serum albumin

had been prepared by salting-out with ammonium sulfate, in others by precipitation with TCA and extraction with acetone. No difference between the half-life of these two preparations was detected. We conclude from our experiments that the serum albumin of newborn rabbits is identical with that of adult rabbits and that its metabolism is not affected by treatment with TCA.

The Comparison of the Hydrolysis of Tosyl-DL-Arginine Methylester by Thrombin and Trypsin. Laskowsky, M. Jr., Purdue University, Lafayette, Indiana.

Sherry and Troll (J. Biol. Chem. 208: 95 [1954]) have shown that thrombin hydrolyzes tosyl-L-arginine methylester (L-TAMe), the best of known substrates for trypsin. Later studies by many workers have shown that while the thrombin specificity towards several substrates is very similar to that of trypsin the activity towards most tryptic substrates is very weak — thus suggesting a higher degree of preferential specificity for thrombin.

Surprisingly both thrombin and trypsin hydrolyze DL-TAMe almost completely. However, of the two, thrombin appears to discriminate a good deal less between the L and D forms i.e. the ratio of k_3L/k_3D is considerably greater for trypsin than for thrombin. Some implications of this result on preferential specificity of thrombin will be discussed.

Biochemical and Physicochemical Studies of Human Plasminogen. Baumgarten, W. and Cole, R. B., Merck Institute for Therapeutic Research, West Point, Pennsylvania.

Plasminogen isolated from human plasma possesses two enzymatic functions when activated with streptokinase, the proteolytic and the fibrinolytic (activator) component. Definite differentiation of these two entities has not been achieved, however, it has been established that at least their precursors, proenzyme and proactivator, prior to conversion with streptokinase possess different stabilities. Similar relationships have also been found in purification studies of crude plasminogen by modified Kline procedure and by subfractionation.

Some Aspects of Thrombolysis in Man. Johnson, A. J., McCarty, W. R. and Tillett, W. S., New York University-Bellevue College of Medicine, New York, New York.

Clinical studies of thrombolysis in man, utilizing streptokinase or streptokinase-plasmin for the therapy of intravascular thromboembolic disease, are complicated by: a) variations in the natural course of disease, and b) an anti-inflammatory effect that may follow clinical use of the enzymes.

In the present studies, experimental trombi were induced in human volunteers. Twenty-four or 48 hours later, streptokinase (SK) was infused under controlled conditions to produce a proteolytic system, *in vivo*, consisting primarily of: (1) circulating SK-plasmin; (2) circulating free SK (or activator); or (3) plasmin plus free SK. The relative efficacy of each system was then tested for its ability to produce sustained lysis of the experimental thrombi.

Thirty-eight thrombi were induced by direct irritation of the intima with a dental broach or by chemical irritation with sodium morrhuate. The position and size of the clots were documented clinically and by venograms. No spontaneous lysis occurred in all (13) controls. In each instance, SK was infused systemically, into a contralateral extremity.

In order to produce fibrinolysis, an initial priming dose was given which was calculated to just neutralize the circulating antibody and inhibitor in each patient. Therefore, additional infused SK was free to produce active fibrinolysis. The additional SK was given in an amount appropriate for the production of one of the three proteolytic systems enumerated above.

The duration of the sustained SK infusion (about 24 hours), and the amount, where determined by following the various biochemical constituents of the fibrinolytic system in the patient's blood, and by clinical and x-ray appraisal of the experimentally induced clot. After clot lysis had occurred, reformation of the clot was prevented, in those patients with sufficient residual plasminogen, by prolonging the SK infusion for an additional 4—6 hours. These studies demonstrate *in vivo* that: (1) Circulating plasmin, without free SK, was ineffective in the

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production of sustained clot lysis; (2) Moderate or large amounts of circulating free SK, without plasmin, depleted the circulating plasminogen (and plasmin) so that there was insufficient substrate for consistent, sustained thrombolysis; (3) Small amounts of circulating plasmin *and* free SK produced consistent, reproducible, thrombolysis and prevented reformation of the clots in all (11) instances.

A Time Lapse Cinematographic Study of Clot Retraction. Ballerini, G. and Seegers, W. H., Wayne State University College of Medicine, Detroit, Michigan.

The film portrays the process of clot retraction as observed in test tubes. The events of two hours duration are reduced to less than one minute. The influence of some of the more commonly known variables on clot retraction are included for education purposes alone. In addition some of the results of more recent investigations are included. The extent of retraction is dependent upon temperature, cell volume, pH, fibrinogen concentration, thrombin concentration, calcium ion concentration, and platelet concentration. Plasma contains dializable material concerned with clot retraction, and this includes glucose, phosphorous, and perhaps other substances. Plasma also contains one or more proteins of importance to clot retraction.

Effects of Human Saliva on Blood Coagulation. Kang, D. H. and Lee, P. H., Yonsei University School of Medicine, Seoul, Korea.

Human saliva possesses the ability to accelerates the prothrombin time and coagulation time of rabbit, dog, and human.

The nature of the accelerating factor is not fully understood, but the work on its purification is quite advanced. It is destroyed by strong alkali as well as strong acid, is unstable to heat above 50° C and insoluble in alcohol, acetone, and ether, indicating the probability of its being protein in nature.

It is also precipitated by a half saturated to a completely saturated ammonium sulfate solution, and it is non-dialysable.

Further study shows it is absorbed by barium sulfate, but not by other adsorbents such as barium carbonate and kaolin. Since it is adsorbed by barium sulfate, and eluated by 3.8% sodium citrate, we were able to collect a relatively large quantity of it.

It's ability to cause a shortening of prothrombin time in either the one-stage or two-stage procedure and clotting time is probably due to its interrelation with thromboplastin.

Buchbesprechungen / Book Reviews / Livres nouveaux

Klinische Methoden der Blutgerinnungsanalyse. (Méthodes cliniques d'étude de la coagulation sanguine). par J. Jürgens et F. K. Beller. Georg Thieme Verlag, Stuttgart, 1959. 408 S. 104 Abb. Preis: 56,— DM.

La première partie a trait à la physiologie et à la pathologie de la coagulation sanguine. Elle constitue une introduction très détaillée à l'étude approfondie des techniques qui font l'objet de ce traité.

Les techniques d'études des facteur intervenant dans la coagulation sont envisagées dans l'ordre suivant: plaquettes, facteurs plasmatiques intervenant dans la prophase de la coagulation (PTA, Hageman, facteurs anti-hémophiliques VIII et IX), facteurs de la première phase (complexe prothrombique), fibrinogène et système fibrinolytique, inhibiteurs de la coagulation.

Ensuite sont détaillées les mesures de la coagulabilité globale, de la rétraction du caillot, la caractérisation de l'héparine.

Le chapitre XV constitue, en fait, une troisième partie consacrée au mode de préparation des réactifs et des facteurs de coagulation.