CHEMICAL, CLINICAL, AND IMMUNOLOGICAL STUDIES ON THE PRODUCTS OF HUMAN PLASMA FRACTIONATION.

XV. THE PROTEINS CONCERNED IN THE BLOOD COAGULATION MECHANISM [1,2]

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The profound biological significance of the clotting of blood needs no emphasis here; the vast number of studies of the process which have been carried out testify sufficiently to its importance, and also show clearly the incompleteness of our knowledge of the underlying mechanisms.[4]

The properties of fibrinogen solutions and of fibrin clots may be profoundly modified in many ways by suitable variations in the conditions of preparation. Thus a wide variety of products is obtainable. In the subsequent papers of this series, the properties and uses of several of these, namely, fibrin clots, fibrinogen plastics, fibrin films, and foams made of fibrinogen and thrombin, will be considered. Here, we shall discuss the properties of certain preparations of protein fractions from human plasma, which may be obtained in active and stable form and which possess specific action on the various aspects of the blood clotting mechanism.

The fundamental feature of this mechanism is the transformation of a solution of the protein fibrinogen into the rigid insoluble fibrin clot. This transformation does not occur spontaneously in solutions of sufficient purity, but is normally brought about by the action of thrombin on fibrinogen. Thrombin, of course, does not occur in significant amounts in normal plasma, but is derived from its precursor, prothrombin. The transformation of prothrombin to thrombin commonly requires the presence of calcium ions, and of some member of the class of activating agents known as thromboplastins. Such a reaction may occur to a very limited extent in circulating blood; but the traces of thrombin which may be so formed are rapidly neutralized by certain constituents of blood plasma which have been termed antithrombins.

A fibrin clot, once formed, disintegrates, even under rigidly sterile conditions, in a period which may vary from hours (or even minutes) to weeks. There is now decisive evidence that this disintegration is the result of enzyme action; the enzyme concerned, commonly designated as fibrinolysin, is apparently a proteolytic substance closely allied to trypsin. Some indications suggest that this enzyme may play an important part in the earlier stages of the coagulation mechanism.

Thus the following discussion will deal with 3 groups of materials:

1. Substances which form the structural basis of the clot: fibrinogen and fibrin.
2. Substances concerned with inducing the process of clot formation: prothrombin, thromboplastin and thrombin, and a special type of "globulin substance" which promotes the clotting of hemophilic blood.
3. Substances which produce dissolution of the fibrin clot: fibrinolysins.

FIBRINOGEN AND FIBRIN

Fibrinogen may be determined in plasma or plasma fractions by electrophoretic studies, in

[1] This work has been carried out under contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Harvard University.

[2] This paper is Number 21 in the series "Studies on Plasma Proteins" from the Harvard Medical School, Boston, Massachusetts, on products developed by the Department of Physical Chemistry from blood collected by the American Red Cross.

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[4] It is unnecessary to give an extensive bibliography of the subject here. We cite, however, a few recent reviews by Eagle (1), Wöhlisch (2), Ferguson (3), Smith (4), Quick (5), and Taylor, Davidson, and Minot (6), which may serve as a guide to the present status of studies in this field.

which it moves with a characteristic mobility intermediate between that of the β- and the γ-globulins (7). In electrophoretic analysis of preparations containing fibrinogen, it is essential that the run be sufficiently long to effect adequate separation between fibrinogen and certain impurities whose mobility differs by as little as 0.5×10^{-5} cm.^{2} per volt per second at pH 7.7 and ionic strength 0.3.

Fibrinogen may also be determined by converting it to fibrin under suitable conditions, and determining the amount of protein nitrogen in the washed fibrin clot (8, 9). In normal citrated human plasma, quantitative clotting may be brought about simply by recalcification; in solutions of the plasma protein fractions, it is necessary to add a small amount of thrombin to secure quantitative clotting of the fibrinogen present.

The fibrinogen content of normal citrated human plasma was determined on pooled plasma samples, obtained from blood collected by the Boston Chapter of the American Red Cross and allotted to the Harvard pilot plant. The fibrinogen content of the 12 citrated samples, indicated in Table I, was determined as percentage of clottable protein. The average fibrinogen content from the 12 runs was 0.25 per cent, or slightly more than 4 per cent of the total protein. It is to be noted that, although 50 cc. of 4 per cent citrate were added to each bleeding, the volume of blood taken from individual donors varied and was not measured. Assuming an average bleeding of 500 cc., of which 55 per cent is assumed to be plasma, one obtains a value of 325 cc. citrated plasma per bleeding. The actual volume of plasma recovered per bleeding has been calculated, from a very extensive series of data, by Dr. H. B. Vickery. Recovery figures range from about 270 to 310 cc. per bleeding, so that a figure of 290 cc. is fairly representative of average recovery. This is 90 per cent of the total value of 325 cc., estimated above. On the basis of these assumptions, the fibrinogen content of uncitrated plasma would appear to be near 0.28 per cent. This figure agrees well with earlier careful measurements on normal plasma (10).

TABLE I

The fibrinogen content of citrated human plasma

Plasma Pool Number	Date	Percentage of fibrinogen
48	July 21, 1942	0.24
49	July 31, 1942	0.25
50	August 6, 1942	0.24
51	August 13, 1942	0.25
52	August 21, 1942	0.24
53	August 28, 1942	0.26
54	September 4, 1942	0.26
56	September 18, 1942	0.25
57	September 23, 1942	0.24
58	October 2, 1942	0.26
59	October 7, 1942	0.26
65	November 17, 1942	0.26
Average		0.25

By the methods of fractionation employed at the Harvard pilot plant, the major portion of the fibrinogen of plasma is concentrated in Fraction I. Moreover, of the total protein of Fraction I, approximately 60 per cent is fibrinogen, representing approximately a 14-fold increase in the proportion of this constituent in this fraction as compared with its concentration in whole plasma. Fraction I may be prepared as a dry sterile powder, which contains both proteins and salts, the latter being citrates or phosphates. In this form, the fibrinogen appears to remain stable for an indefinite period. On addition of distilled water, the powder readily redissolves, and the resulting solution is promptly clotted by the addition of thrombin. In the absence of added thrombin, the solution remains clear and does not clot for a period which is at least several hours and may be as long as 3 or 4 days. For most clinical applications, further purification is unnecessary; but by suitable further fractionation, preparations may be regularly obtained which contain 85 per cent or more of clottable protein. Determinations of fibrinogen content by prolonged electrophoresis and by the clotting method give very good agreement both for Fraction I and for more highly purified fibrinogen (Table II). In the instance of whole plasma, routine electrophoretic analysis yields a value for fibrinogen slightly higher than that obtained by the clotting method, owing to incomplete resolution of the fibrinogen peak from the slowest components in the β-globulins.

It is important also that Fraction I should contain as large a percentage as possible of the total fibrinogen in plasma. Under the best

TABLE II

Comparison of Tiselius analysis with clottable nitrogen

	Fibrinogen as determined by clottable nitrogen	Fibrinogen as determined by electrophoresis
	per cent	*per cent*
Preparation No. 27 III		
Fraction I	66.6	66
1st reprecipitation	76.1	75
2nd reprecipitation	84.4	84
Preparation No. 39		
5th reprecipitation	90.1	90

conditions so far worked out, the total yield in Fraction I approximates 75 per cent[5] (Table III).

The fibrinogen molecule is larger and far more elongated than any other plasma protein molecule. Its high asymmetry is shown by the fact that its solutions are highly viscous and show well-marked double refraction of flow.[6] These properties of fibrinogen, first recognized by other investigators, have been confirmed and extended by studies made in these laboratories. The molecular weight of fibrinogen is of the order of 500,000; the molecular length [7] is of the order

TABLE III

Fibrinogen concentration and percentage recovery of fibrinogen in whole plasma, found in Fraction I

Run no.	Fibrinogen	Recovered
	per cent	*per cent*
89	61.0	84
90	59.1	73
91	60.4	75
Average	60.2	77

of 900 Å. Thus the molecule may actually be regarded as a kind of miniature rod or fiber. Recent x-ray studies (12) confirm and amplify this conception, indicating that fibrinogen and fibrin both possess an underlying structural pattern analogous to that of the fibrous proteins, keratin and myosin. These studies indicate no fundamental difference in the detailed molecular pattern of fibrinogen and fibrin; the process of clotting appears rather to involve a linkage of the long fibrinogen molecules into far longer needle-like microcrystals. Elongated needles of fibrin have indeed been clearly seen directly by microscopic or ultramicroscopic observation in fibrin clots (13 to 15). Studies of the double refraction of oriented fibrin fibers (16) have also furnished clear evidence for the presence of elongated rod-shaped molecular structures, similar to those found in fibers such as those of muscle or tendon.

The nature of the transformation of fibrinogen to fibrin, as induced by thrombin, is still obscure. Highly suggestive, however, are the recent experiments of Chargaff and Bendich (17) who have shown that such substances as ninhydrin and the sodium or potassium salts of 1,2-naphthoquinone-4-sulfonic acid, and 1,4-naphthoquinone-2-sulfonic acid produce a coagulation of fibrinogen which is strikingly similar to the natural fibrin clot. It is very suggestive that nearly all these substances can oxidize amino acids and peptides, containing free amino groups, with simultaneous decarboxylation. It is still too soon to judge whether a similar mechanism is involved in the action of thrombin on fibrinogen; the attempts of Chargaff and Bendich to detect a liberation of CO_2 during this process led to inconclusive results. We may hope, however, for a deeper understanding of the chemistry of the process in the near future.

Some of the diverse uses of the fibrin clot, and the modified forms in which it may be obtained, are discussed in Papers XVI to XXI of this series (18 to 23).

PROTHROMBIN

The first prothrombin preparations of high potency were obtained by Mellanby (24), the initial step in his process being dilution of the plasma, and precipitation of prothrombin by adjustment of pH to 5.3. His work indicated clearly that prothrombin is a euglobulin with a point of minimum solubility close to this pH. Prothrombin is very readily adsorbed by many reagents, such as the insoluble hydroxides and silicates of magnesium and aluminum. Adsorption and elution, together with other procedures,

[5] We may also call the reader's attention to a recent simple method for preparing human fibrinogen of high tensile strength, described by Neurath, Dees, and Fox (11).

[6] See Wöhlisch (2), pp. 277–293; and for the general significance of these properties, see Paper I of this series (7).

[7] From unpublished double refraction of flow measurements by I. H. Scheinberg in this laboratory.

have been skillfully employed by Seegers (25) in obtaining the most active prothrombin preparations yet achieved. The material obtained by Seegers was a protein containing about 4 per cent carbohydrate. Its solubility behavior was similar to that indicated by Mellanby's work, and it was found to be very unstable in solutions acid to pH 4.8, or alkaline to 9 or 10. Like thrombin, it was nondialyzable.

In the process of fractionation employed in these studies (7), prothrombin was found to be concentrated in the proteins of Fraction III-2. Its activity in this fraction, expressed in prothrombin units [8] per milligram of protein, is generally from 12 to 15 times as great as in the original plasma, and in favorable cases is considerably higher than this.

The possibility of injecting concentrated prothrombin solutions into patients suffering from prothrombin deficiency has frequently been suggested as a therapeutic measure, for while certain of such deficiencies are frequently and readily treated with vitamin K, cases occur in which such therapy is without effect, notably in the presence of reduced liver function. The risk of intravascular clotting from prothrombin injections must, of course, be considered a very serious one. The preparations of prothrombin obtained by us are as yet to be considered unsafe for intravenous use. They are unstable from two standpoints: first, the preparation rapidly loses activity unless the prothrombin is converted to the far more stable thrombin; secondly, spontaneous formation of small quantities of thrombin occurs without the addition of any converting agent. Work aimed at the elimination of these drawbacks (which are shared by practically all prothrombin preparations hitherto reported) is now in progress.

[8] We have followed the definition of Seegers, Smith, Warner, and Brinkhous (26) who state "One unit of prothrombin is that amount which, when completely converted into thrombin will clot 1 cc. of fibrinogen solution in 15 seconds." The concentration of fibrinogen has little influence on the clotting time provided it lies in the range 0.3 to 1.0 per cent. However, the type of fibrinogen employed, and the method of its preparation, may considerably influence the results obtained. Also the criteria of clot formation, used in evaluating clotting times, have been chosen differently by different workers. Therefore, the value of the prothrombin unit, by the above definition, is probably not the same in different laboratories; although highly consistent results can be attained by the workers in any one laboratory, using a carefully standardized technic.

The unit of thrombin is determined in exactly the same way as that of prothrombin, except that the solution to be tested is added directly to the fibrinogen solution, without any attempt to convert the prothrombin present at the time of addition.

THROMBIN

A variety of uses has been found (18, 21, 22) for the prothrombin present in Fraction III-2 when converted into thrombin. Bovine thrombin of very high potency has been prepared by Seegers (25 to 27) and by Milstone (28). The methods of purification, although in many ways radically different, yield products of comparable activity. The thrombin of Seegers appears to be somewhat the more potent of the two. The chemical properties of thrombin are in many ways similar to those of prothrombin. Its pH of minimum solubility, however, is reported to be more acid (25); and it is distinctly more soluble than prothrombin at almost all pH values and salt concentrations. Like prothrombin, however, it is an euglobulin—that is a protein which at or near its isoelectric point is only slightly soluble in water, but is much more soluble in dilute salt solution. The work of Milstone (28) has shown that thrombin is not so readily salted out as prothrombin. Thrombin is soluble in 0.45 saturated ammonium sulfate solution, whereas crude prothrombin is not. Thrombin is also considerably more stable than prothrombin, particularly in acid solutions.

A much less highly purified, but extremely simple and clinically effective preparation, was obtained by Parfentjev (29) who fractionated rabbit plasma with ammonium sulfate, and obtained a fraction high in thrombic activity, which retained its activity in solution over a long period of time. Since the original plasma, before fractionation, contained little or no thrombin, it is probable that the fractionation procedure effected the conversion of prothrombin to thrombin, in addition to separating the resulting thrombin in one of the fractions obtained. The mechanism of the effect remains unexplained, but Parfentjev's findings were confirmed and extended by Taylor, Lozner, and Adams (30); and Lozner, MacDonald, Finland, and Taylor

(31) showed "that rabbit thrombin is of distinct value in the control of hemorrhage from *small wounds*." Similar preparations have now been obtained from the plasma of man and of several other animals in addition to the rabbit (32).

The human thrombin preparations obtained in these laboratories, by the conversion of the prothrombin in Fraction III-2 with human thromboplastin of placental origin, consist predominantly of β-globulin, to the extent of about 75 per cent as determined by electrophoresis. It is not yet possible to state, however, that thrombin is itself a β-globulin, since thrombin certainly makes up only a small part of the total protein present in the preparation. Solutions of this material retain their activity for several days in the cold. Thrombin solution may be filtered into a sterile container, frozen, and dried. The resulting white powder remains stable over a protracted period of time [9] when preserved in the dry state and redissolves readily on the addition of saline solution, forming a slightly opalescent liquid of high thrombic activity. The activity is of the order of 15 to 20 units of thrombin per milligram of protein in the dry powder. Whereas this preparation has thus far less activity per milligram [10] than the highly purified preparations of Seegers and Milstone, its potency lies far above minimal requirements for clinical use, and the impurities present in our preparation, as well as the thrombin itself, are all proteins of human origin, so that there is no obvious practical advantage to be gained by further purification for clinical use.[11]

THROMBOPLASTIN

The conversion of prothrombin to thrombin is ordinarily very slow and incomplete except in the presence of calcium ions and of an activating material of the class commonly termed thromboplastin (or thrombokinase).[12,13] It now appears clear that certain thromboplastins, at least, are lipoproteins. The one most thoroughly studied is that obtained from the lungs of cattle and investigated by Chargaff and his collaborators (33 to 36). The active protein material is readily precipitated at low salt concentrations in the neighborhood of pH 5.1. The lipids could be extracted from the protein with a mixture of boiling alcohol and ether; the lipid-free protein, after this extraction, showed no thromboplastic activity. The lipid components were shown to contain a number of fatty acids, both saturated and unsaturated; also a large amount of choline and a small amount of ethanolamine, together with other unidentified bases (possibly serine). The union between the lipid and the protein in the material freshly obtained from lung appears to be very firm. Recent electrophoretic studies (33 to 36) have shown that the thromboplastin in solution migrates with a single boundary in the Tiselius apparatus at pH 7.4 and pH 8.8. A small fraction of the preparation, which contains nucleic acid, moves with a different velocity and can thus be separated by electrophoresis. The nucleic acid fraction is apparently not necessary for the thromboplastic activity.

The thromboplastic protein is most effectively separated by sedimentation in a preparative ultracentrifuge, employing centrifugal accelerations about 30,000 times gravity (33 to 36); the precipitate so obtained redissolves readily in a borate buffer at pH 8.6. Its sedimentation constant is reported (35) to be near 330 Svedberg

[9] Recent tests on one preparation show no loss of thrombic activity on heating for 115 days at 50° C. Further thermal stability tests on this and other preparations are being carried out.

[10] As explained in footnote 8, the units employed by us may not be strictly comparable with those of other authors. We are inclined to believe that our unit is larger than that of Seegers, but a precise comparison of the two has not yet been carried out.

[11] The thromboplastin employed (see the discussion below) in converting prothrombin to thrombin is obtained from human placentas, thus no protein of non-human origin is involved in the preparation.

[12] Milstone (28) has recently made the striking observation that concentrated prothrombin solutions are slowly converted to thrombin in 0.1 saturated ammonium sulfate at pH 5.4. The solution, after dialysis against ammonium sulfate solution in the cold, was kept one week at 20°, when conversion was complete, and then stored at 1°. No added activator was required, and the addition of oxalate did not retard the process of conversion. As yet, we have not found it possible to employ a "spontaneous" conversion of this type in the preparation of human thrombin.

[13] It is impossible in this place to give any adequate discussion of the extensive literature concerning thromboplastin. The reviews cited (1 to 6), especially Chapter V of the monograph by Quick (5), supply extensive references on the subject.

units, and its molecular weight to be close to 170 million; it contains approximately 8 per cent nitrogen and 1.4 per cent phosphorus. The thromboplastic activity is so great that as little as 0.008 μgm. markedly accelerates the clotting of 0.1 cc. of rooster plasma; the preparations also show marked phosphatase activity. In its large size and high lipid content, this material appears similar to the "microsomes" obtained by Claude (37, 38) from the cytoplasm of many tissues, although as yet no thromboplastic activity has been reported for the latter.

The thromboplastic lipoprotein of lung can be made to dissociate by the action of heparin (33 to 36), but in that this report is not intended to treat the very numerous communications dealing with the function of heparin in prevention of coagulation, the possible implications of this finding lie outside its province.

A tissue globulin having high thromboplastic activity has been obtained from human placentas (39); it is insoluble at pH 5.2, and soluble at pH values alkaline to 7. It appears, however, to be a very large molecule, and is removed by passing the material through a Sharples centrifuge. Thromboplastic activity is rapidly lost in acid or alkaline solutions, and more slowly by oxidation or by aging, especially in the presence of fresh serum. The properties of this material are similar to those of the thromboplastic protein from lung; but chemical analyses of the placental protein are not yet available. It is reported to be of value in the treatment of hemophilia (39).

This placental thromboplastin has been used in our laboratory to convert human prothrombin to thrombin in the presence of calcium ions. It has proved to be a highly efficient converting agent, and is stable for a period of at least several weeks if kept at 0 degrees with a small amount of tricresol as a preservative. Although its activity is not quite so high as that of some thromboplastins of animal origin, it has the great advantage of containing only human material, and therefore introduces no heterologous protein when added to human plasma protein fractions.[14]

[14] We are indebted to Miss K. Fahey of the Children's Hospital, Boston, for preparing this material and supplying it to us.

THE "GLOBULIN SUBSTANCE" WHICH PROMOTES THE CLOTTING OF HEMOPHILIC BLOOD

In 1937, Patek and Taylor (40) obtained from normal human plasma, by dilution and acidification, a globulin fraction which greatly accelerated the clotting of hemophilic blood. The most active fractions were those precipitated at pH 5.5 to 6.0. Similar fractions prepared from hemophilic blood were inactive or very weakly active. The active factor was thermolabile, and soluble in isotonic saline. An active preparation could also be obtained by the removal of salts by dialysis from normal human plasma (41). The euglobulin so obtained was active in reducing the coagulation time of hemophilic blood, both *in vitro* and *in vivo*. The active factor was not identical with prothrombin or thrombin. It also appears different from tissue thromboplastins, since it passes readily through a Berkefeld filter and does not shorten the clotting time of normal blood.

We are indebted to Dr. F. H. L. Taylor and his associates at the Thorndike Memorial Laboratory of the Boston City Hospital for testing the plasma fractions separated at the Harvard pilot plant for the presence of this clotting factor. It was found that the active factor was present to a very large extent in Fraction I. The rest was almost all concentrated in Fraction II + III, and could be further subfractionated in Fraction III-2. Prothrombin, as we have seen, is also concentrated in this fraction; but the reported data (40, 41) for the fractions which shorten the clotting time of hemophilic blood suggest that the active component is not so labile as prothrombin.

FIBRINOLYSIN

That a slow dissolution of the fibrin clot occurs, even under completely sterile conditions, has long been known (42). The time required for this dissolution, however, varies greatly in different clots; it may vary from a few hours, or even less, to 2 or 3 weeks. A typical clot obtained from Fraction I, formed under sterile conditions in the presence of a minute amount of thrombin, dissolves in the course of 1 to 10 days, the time required for dissolution depending on pH, temperature, and other factors. A sensitive

optical method for following the course of fibrinolysis by relative turbidimetry, using the Evelyn photoelectric colorimeter, has recently been described by Ferguson (43, 44).

The speed of fibrinolysis is greatly increased if large amounts of Fraction III-2, or of the thrombin prepared from this fraction, are added to the clot. Thus, the addition of large amounts of the human plasma fraction containing thrombin accelerates both the formation of the clot and its subsequent dissolution. When very large amounts of Fraction III-2 are added, relative to the amount of fibrin in the system, lysis may begin within a few minutes after the clot is formed, and be complete within half an hour. There is good reason to believe, however, that the lytic factor is not identical with prothrombin or thrombin.[15]

The fibrinolytic power is remarkably enhanced if plasma or certain plasma fractions are well shaken with chloroform, and the mixture allowed to stand for a period of from one hour to several days. On removal of the chloroform, the resulting serum is found to dissolve fibrin clots with extraordinary rapidity. The earlier work in this field has recently been summarized by Tagnon (45), who has also shown that lysis of fibrinogen, as well as of fibrin occurs, so that chloroform serum, even when it contains small amounts of thrombin, may not cause clotting of fibrinogen but simply a breakdown of the fibrinogen molecule which renders the solution incapable of clotting when more thrombin is added. The activity of the activated enzyme is inhibited by plasma fractions containing high concentrations of prothrombin, but there is as yet no proof that prothrombin is itself an inhibitor. The action of the enzyme is definitely proteolytic, since it is capable of digesting fibrinogen, fibrin, gelatin, and casein, as indicated by the progressive formation of non-protein nitrogen when it acts upon these substrates (46, 47). It also causes a progressive decrease in the viscosity of gelatin. Its optimum activity is at pH 7.4, and it is inactivated by heat.[16]

Tagnon (45, 46) has emphasized the fact that considerable amounts of thrombin may be found in chloroform treated plasma, previously freed of calcium and platelets, or other sources of thromboplastin. He believes that the enzyme itself may function as the active agent for converting prothrombin to thrombin, although the thrombin so formed may be subsequently destroyed on prolonged standing, through the proteolytic activity of the enzyme. In recent years, Ferguson (48) has independently been developing a similar view, which assigns to a proteolytic enzyme of plasma—termed by Ferguson serum-tryptase—a key position in the initiation of the clotting process. In this connection, the demonstration by Eagle and Harris[17] that crystalline trypsin converts prothrombin to thrombin is certainly highly suggestive.

It is impossible to give at present any final evaluation of these conceptions. If the enzyme functions as the essential agent for converting prothrombin to thrombin, then it must be released from an inactive form by some natural activator which performs the same function which is subserved by chloroform *in vitro*. While the blood remains fluid as it circulates in the body, the action of this activator must be held in abeyance. Thus, a still unknown mechanism must be postulated, and its nature explored, if the proteolytic enzyme theory of clotting is to be accepted. In this communication, however, we are not directly concerned with the theory of clotting, but rather with the preparation of active and stable materials, which act on different phases of the blood clotting mechanism, and which are susceptible of possible clinical use.

SUMMARY

In the course of the large scale fractionation of human plasma, preparations containing certain of the proteins concerned in the blood co-

[15] Evidence bearing on this point is given by Wöhlisch (2), pp. 363–368.

[16] Determinations on our human plasma protein fractions by Dr. Taylor and his associates, together with measurements from this laboratory, indicate that the enzyme precursor which can be activated by chloroform is found in several different plasma protein fractions—some of it in Fraction I, and larger amounts in Fractions II + III and IV. On subfractionation of II + III, most of it is concentrated in Fraction III-2; on subfractionation of IV, most of it goes into Fraction IV-1. The distribution of the enzyme among different fractions appears to be somewhat variable from one preparation to another.

[17] See Eagle (1), p. 106.

agulation mechanism have been obtained. Outstanding among these are (*1*) fibrinogen, which is concentrated in Fraction I, which can be further purified when this is desired for special purposes, (*2*) thrombin, prepared from the prothrombin which is concentrated in Fraction III-2, by conversion with human placental thromboplastin. Fraction I, purified fibrinogen, and thrombin have all been prepared in dry, stable and sterile form; and they serve as starting materials for the making of a series of products, the properties and uses of which are described in subsequent papers of this series. The properties of certain other substances, related to the clotting of blood and the dissolution of blood clots, are also discussed; and the chemical properties and functions of all these substances are briefly considered.

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