THE PHARMACY OF BLOOD, ITS PRODUCTS AND SUBSTITUTES*

THE BLOOD CLOTTING MECHANISM

BY PROFESSOR J. E. JORPES, M.D.

Chemistry Department, Karolinska Institutet, Stockholm, Sweden.

WHEN blood clots one of the plasma proteins, fibrinogen, is transformed to a gel, fibrin. Although fibrinogen makes up only one thirtieth of the plasma protein, on clotting it gives the blood a solid consistency, sufficiently strong to prevent further bleeding in case of smaller lesions.

FROM WILLIAM HEWSON TO H. P. SMITH

The first description of fibrin is to be found in Marcello Malpighi's *Dissertatio de Polypo Cordis* in the year 1666[1]. When the coloured matter was washed away from the clotted blood a whitish fibrous substance remained. Emboli from the heart behaved in a similar way. About 100 years later Hewson[2,3] clearly differentiated "the coagulable lymph" which clotted spontaneously in the air at room temperature from "the clottable matter of serum" which like egg-white clotted at first on heating.

Hewson demonstrated that blood without contact with the air remains fluid for a long time in a suspended vein. He found that blood, to which sodium sulphate had been added, retained its clotting ability for a time on diluting afterwards with water; this was the first practical way of preserving blood for experimental purposes. Hewson was the first to point out that fluid blood deprived of red corpuscles, that is the plasma, contained all constituents necessary for the clot formation.

In Hewson's time the ideas about the fluidity of the blood were very simple. The most common view was that the motion of the blood separates the fibrin particles and the blood corpuscles, which were thought to participate in the clot formation. The escape of carbon dioxide, known since 1783 to occur in the blood, or of ammonia, when the blood leaves the vessels, was also thought to induce coagulation. In the middle of the previous century, Richardson, an English physician, was an ardent defender of the theory that ammonia kept the fibrin in solution in the circulating blood. Joseph Lister[4] disproved this view in his Croonian Lecture of 1863.

Andrew Buchanan[5] in Glasgow was the first to make observations indicating a catalytic reaction in the clotting process. In 1845 he showed that ascitic and hydrocele fluid clotted when an extract of clotted fibrin was added to them. The blood clot itself, muscle, and other tissues had a similar action when added to blood. According to Buchanan there is a resemblance between the clotting of blood and the clotting of the milk by rennin.

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The enzymatic theory of coagulation was put on a sound basis by Virchow[6]. The founder of cellular pathology took particular interest in blood coagulation. In 1845 he made one of his great contributions to medicine when he explained that pulmonary emboli are clots from disrupted thrombi of the peripheral veins. Our main conceptions of the enzymatic processes leading to coagulation originate from his institute. It was here that Alexander Schmidt was educated. In 1861 Schmidt's new theory about coågulation was presented to the Berlin Academy of Science. His theory postulated that the coagulation proceeds in two phases. In the first phase thromboplastic substances are liberated from blood cells and tissues, which are capable of transforming a plasma component, prothrombin, into the active clotting enzyme thrombin. In the second phase the thrombin produces insoluble fibrin from its soluble precursor, which Virchow in 1847 named fibrinogen. Thus the terms thrombin, prothrombin, thromboplastic substances and fibrinogen all have their origins in the school of Virchow.

A new component was introduced in the discussion, when Arthus and Pages[7] in 1890 found calcium salts to be necessary for coagulation. By adding decalcifying agents such as oxalate, fluoride or citrate to the blood a new way of keeping the blood fluid became available.

Olof Hammarsten[8] in Uppsala proved some years later that calcium is necessary only in the first phase of coagulation, but not for the action of thrombin.

The classical theory of Alexander Schmidt as demonstrated in Figure 1 was supplemented in the years around 1905 by the theory of Spiro, Fuld and Morawitz (see Morawitz[9]).

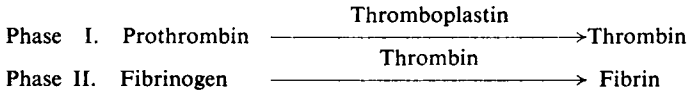

FIG. 1. The classical theory of blood coagulation.

Particular attention was paid to the study of the lipid component of thromboplastin by Bordet in Belgium and Howell in Baltimore. In this study Howell and MacLean discovered heparin in 1916 (see Jorpes[10]). Except for this, research on blood coagulation went into a 30-year hibernation period.

PROTHROMBIN ENTERS CLINICAL MEDICINE

In the nineteen-thirties interest in blood coagulation was given a new impetus. At the Iowa State University H. P. Smith introduced the principle of studying the different stages in the coagulation process on a quantitative basis with products as pure as possible. Thus the first reliable methods of determining prothrombin were elaborated. These are a two-stage method by Smith and others[11] and a one-stage method by Quick[12] in Milwaukee. A prothrombin deficiency was now found to be the cause of cholæmic bleeding[13], the hæmorrhages due to deficiency of vitamin K[14] and the bleeding tendency during the neonatal period[15].

The discovery of vitamin K in 1934 by Henrik Dam[16] in Denmark, and the work done by Charles and Scott[17] at the Connaught Laboratories of the University of Toronto on heparin in 1933 followed by the elucidation of its chemical nature in 1935[18] gave two new therapeutic possibilities of the utmost importance for medicine. In 1938 vitamin K was introduced as a prophylactic agent against the hypoprothrombinæmic bleeding tendency in obstructive jaundice and during the neonatal period. At about the same time heparin was found to be an efficient remedy for thrombo-embolic diseases. Nothing in the history of coagulation has exerted such a stimulating influence as these happenings particularly after Karl Paul Link's work. From 1940 to 1942 he studied the hæmorrhagic agent causing sweet-clover disease in cattle. The agent, named dicumarol, soon proved to be an excellent antithrombotic remedy (see Jorpes[19]). In his introduction to the number of the *British Medical Bulletin* (1955, **11**, No. 1), devoted to blood coagulation and thrombosis, Macfarlane points out that for every patient who dies as a result of deficient coagulation, there are thousands who die of thrombosis. Hence the importance of this new efficient specific therapy against thrombo-embolic disorders. Prothrombin now becomes a key substance in clinical analysis, almost as important as hæmoglobin.

New Coagulation Factors are Discovered

Thus, in the nineteen-thirties prothrombin was the central topic under discussion. During the next decade the main interest was focused on newly discovered coagulation factors concerned with prothrombin activation.

First consideration must be given to some early findings about the cause of hæmophilia. In 1911 Addis[20] found that a fraction of normal plasma accelerated the clotting of hæmophilic blood. He considered prothrombin to be the active component in it. During the period 1936 to 1937 Patek, Taylor *et al.*[21,22] at the Thorndike Memorial Laboratory in Boston were working on a plasma fraction found to be lacking in hæmophilia and hence called the antihæmophilic globulin. The globulin fraction, however, did not prove to be more useful than whole blood for the purpose of correcting the clotting deficiency in hæmophiliacs and no practical therapeutic results came from the work. These findings focused the interest upon the plasma proteins at a time when the cause of hæmophilia was considered to be exclusively a platelet deficiency.

Much more attention was paid to the discovery in 1943 by Paul Owren[23,24] in Oslo of a new factor, the absence of which causes a kind of hæmophilia, called by Owren parahæmophilia. He found a case with a congenital bleeding tendency in whose blood all known components of the coagulation system, fibrinogen, prothrombin and calcium, were present in normal concentrations and the thrombocytes seemed to function as in normal blood. Owren called the new component factor V, thereby disregarding Patek's and Taylor's discovery of the antihæmophilic globulin some 6 to 7 years earlier. Owren's discovery of factor V, later called proaccelerin and in its activated form factor VI or accelerin,

is unique at least in one respect. It was made by a young physician in a medical clinic, practically without laboratory facilities, and this during the difficulties of the German occupation of Norway.

At that time, Astrup[25] in Copenhagen made observations indicating that not thrombin but thromboplastin is formed during the autocatalytic reaction initiating coagulation, the active thromboplastin being derived from an inactive "prokinase." This corresponds very closely to our present-day conceptions.

Simultaneously with Owren, Quick[26], in 1943, found that a labile plasma component, assumed by him to be a part of a prothrombin complex and called prothrombin A, enters into the coagulation system. In fact Smith[27] and his colleagues of the Iowa group had already in 1939 pointed out that there is a "prothrombin convertibility factor" occurring in different concentrations in the plasma of different mammalian species. This effect of plasma was studied more closely by Seegers[28] and colleagues in 1947. As a consequence of these studies Seegers prefers to use the term accelerator globulin or plasma Ac-globulin instead of factor V or proaccelerin.

In several laboratories it was observed at the same time that an additional factor, other than calcium, thromboplastin and factor V, was essential for a complete and rapid conversion of prothrombin into thrombin. In analysing the different prothrombin preparations Owren[29] in 1947 had already drawn attention to a co-factor of proaccelerin, which was essential for coagulation. Later he called the factor proconvertin. Alexander[30,31] and colleagues in Boston, who made the most extensive study of this plasma component, named it "serum prothrombin conversion accelerator," SPCA. In 1951, this component was shown by Koller, Loeliger and Duckert[32] in Zürich to be closely related to prothrombin, being taken up by ordinary prothrombin adsorbents and strongly reduced in quantity by the action of dicoumarol. Koller named the component factor VII.

An interesting member of the series of coagulation factors is a new antihæmophilic protein. In 1947 Pavlovsky[33] in the Argentine observed that normal clotting capacity of the blood of a hæmophiliac could be restored by the blood from another hæmophiliac, suggesting that the hæmophilia of these two patients must have been caused by the deficiency of two different factors. Similar observations were made in other countries. In Switzerland, in 1950, Koller *et al.*[34] found a family with a hæmophilia-like disease, the "Moëna anomaly" which behaved in a similar manner. In 1952 Aggeler *et al.*[35], in describing a case of hæmophilia, showed that the coagulation deficiency was due to a lack of a well-defined plasma protein differing from the ordinary antihæmophilic globulin. It was called "plasma thromboplastin component, PTC."

Biggs, Douglas and Macfarlane[36–38] found in 1952 and 1953 a similar coagulation deficiency in seven patients. They coined the name Christmas-factor for the essential plasma protein and Christmas-disease for this form of hæmophilia. Not less than 9 different names have been suggested by different authors for this plasma component.

Koller[39] has recently suggested the use of numbers for the different components entering into the coagulation, fibrinogen being factor I, prothrombin factor II, thromboplastin factor III, calcium factor IV, proaccelerin and accelerin factor V and VI, convertin factor VII, the antihæmophilic globulin factor VIII and the Christmas-factor, factor IX. Koller himself added still another component, factor X, to the series. The synonyms suggested for the new coagulation factors, from 5 to 13 for each one of them, are presented by different authors of recent monographs[40–42].

In addition to the two types of hæmophilia caused by the lack of factor VIII and factor IX respectively, a third type was described by Rosenthal[43] in New York in 1953. The missing plasma component was called plasma thromboplastin antecedent or PTA. This form of hæmophilia occurred with equal frequency in males and females.

The two first mentioned forms of hæmophilia were named hæmophilia A and B by Cramer *et al.*[44] and by Soulier[45].

All the factors presented here, except fibrinogen, are active in the first phase of the coagulation. According to the modern view the following clotting factors take part in the coagulation (Fig. 2, Koller[39]).

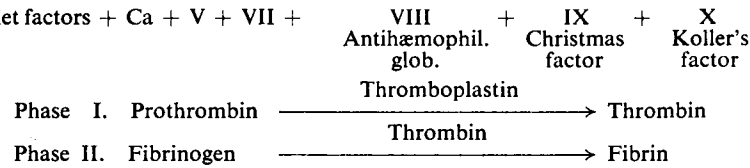

FIG. 2

SOME PROPERTIES OF THE COAGULATION FACTORS AND THEIR FATE DURING COAGULATION

Except for the peptides split off during coagulation all the fibrinogen is transformed into fibrin.

The antihæmophilic globulin (VIII) behaves in many respects like fibrinogen. It is precipitated at 25 per cent. saturation with ammonium sulphate and follows the fibrinogen in fraction I in Cohn's fractionating procedure 6 for the separation of plasma proteins. It is not adsorbed on the ordinary prothrombin adsorbents and not reduced during dicumarol treatment. As shown conclusively by Brinkhous *et al.*[46] in using hæmophilic dogs it disappears during coagulation. According to Tocantins and Seegers the serum still contains plenty of antihæmophilic globulin, although bound to a lipid inhibitor, which can be removed with ether.

The Christmas-factor (IX) on the other hand is precipitated at 35 to 50 per cent. saturation with ammonium sulphate, it precipitates in fraction IV of the Cohn fractionation, migrates with the β-globulin and is taken up by the prothrombin adsorbents. After coagulation it is found in the serum, where it is fairly stable on storage.

Factor V and VI, the Ac-globulin, is stable in the plasma only for a limited time and disappears during coagulation. It is not adsorbed on calcium phosphate, asbestos or aluminium hydroxide and is not influenced by dicumarol treatment.

Factor VII behaves almost exactly like prothrombin. It is, however, not consumed during coagulation and is fairly stable in serum.

What Happens During Coagulation?

Hewson was fully aware of the fact that contact with foreign surfaces initiates coagulation and Alexander Schmidt spoke about the liberation of activating thromboplastic substances from the cells and tissues. Bizzozero[47] in 1882 observed that thrombocytes accumulate around the lesion, when a thrombosis is produced mechanically in the mesenteric vessels. In 1912 Bordet and Delange[48] showed that the thrombocytes liberate thromboplastic substances. Consequently these cells were considered to be the main source of the thromboplastin. In the following years the clot promoting agents contained in tissue thromboplastin were

TABLE I

THE PROBABLE SEQUENCE OF REACTIONS AND THE TIME NECESSARY FOR THE TWO PHASES OF THE NORMAL COAGULATION ACCORDING TO BIGGS[42]

	Reactions involved	Product formed	Time occupied by the reaction
Phase I	Contact with a foreign surface Platelets, antihæmophil. glob. Christmas-factor (IX) and calcium	Intermediate product of thromboplastin	2–5 min.
	Factor V and VII and calcium	Thromboplastin	8–10 sec.
	Prothrombin and calcium	Thrombin	2–5 sec.
Phase II	Fibrinogen	Fibrin	2–5 sec.

studied particularly by Chargaff and his colleagues[49]. They showed that the thromboplastin preparations from lungs, brain and other organs were high-molecular complexes or aggregates, containing protein, lipids nucleic acids and carbohydrates. Using thromboplastin labelled with radioactive phosphorus, Chargaff demonstrated that chemical combination between thromboplastin and prothrombin is improbable. With the introduction of the silicone technique[50] it became possible to study the platelet morphology and interaction of platelets. However, lately it has been found that the plasma thromboplastin is not liberated fully-formed from the thrombocytes but that it arises as a product of an interaction between plasma factors and accelerators from the platelets. The question arises to what extent the contact with foreign surfaces activates the plasma factors and to what extent the activated components react with the thrombocytes. Thus Brinkhous[51] in 1947 clearly demonstrated that a plasma factor, lacking in hæmophilia, is required for platelet utilisation during the normal coagulation. The factor was suggested by him to be a thrombocytolysin.

The interaction between the plasma factors and factors derived from the platelets has been the main topic of study during the last years. Blood, lacking some of the antihæmophilic factors, or with some platelet deficiency, has offered the best material for these studies.

In Table I and Fig. 3 the views concerning the coagulation mechanism held at present by two well-known authors, Rosemary Biggs[42] and Paul Owren[40] are summarised.

It is common knowledge that addition of brain extract, that is, "extrinsic thromboplastin," to blood or plasma causes rapid coagulation. According to Biggs, Douglas and Macfarlane[37] during normal clotting a substance forms which is highly active, much more so than the tissue thromboplastin. Thus in the presence of calcium the activators liberated from the thrombocytes react with the antihæmophilic globulin and the Christmas-factor to give an intermediate product usually called "intrinsic thromboplastin." According to Biggs, factors V and VII are, like calcium assumed to participate in the final thromboplastin formation. These parts of the coagulation process are the most time-consuming ones, the thrombin and fibrin formation like many other enzyme reactions being almost instantaneous. Biggs, Douglas and Macfarlane[38] developed a thromboplastin generation test by means of which the hæmophilias A and B and deficiencies of the thrombocytes can be recognised.

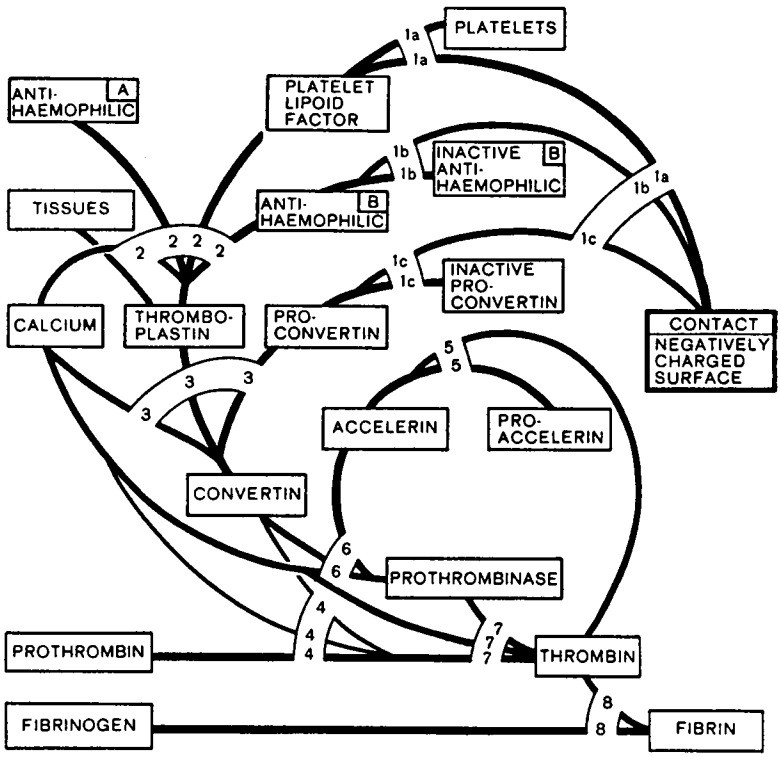

FIG. 3. Blood coagulation theory of P. A. Owren.

OWREN'S SCHEME OF COAGULATION, 1954

In Owren's scheme factor VI and VII participate together with thromboplastin in the transformation of prothrombin to thrombin. Koller and colleagues[52] like Biggs do not consider these factors necessary for the action of the plasma thromboplastin. However, the two schemes do express the same thing although in different words. Owren's prothrom-

binase is a fully correct name for the final thromboplastin because peptides are split off enzymatically from prothrombin during its activation.

As to the different factors deriving from the platelets and from the plasma, participating in the thromboplastin formation, Seegers[53] gives the following classification:

TABLE II

CLASSIFICATION OF PLATELET AND PLASMA CO-FACTORS

From the platelets:	
Platelet factor 1	Accelerator factor (platelet-AcG)
Platelet factor 2	Fibrinoplastic factor (thrombin-fibrinogen)
Platelet factor 3	Threone component (thromboplastin factor)
Platelet factor 4	Antiheparin factor
In the plasma:	
Platelet co-factor I	Threone component (antihæmophilia factor)
Platelet co-factor II	PTC (plasma thromboplastin component)

Thus not less than four factors are obtained from the thrombocytes (see also Ackroyd[54]). The two co-factors occurring in plasma, the "platelet co-factor I" or the "threone component" and the "platelet co-factor II" or Aggeler's "plasma thromboplastin component"[55], would have been easier to recognise if they had been named the antihæmophilic globulin (VIII) and the Christmas-factor (IX) respectively.

According to Seegers[56,57] the platelet factor I functions like the accelerator globulin and can in fact restore the Ac-globulin deficiency of stored, oxalated human plasma. Likewise Owren[58] recently reported about the proaccelerin activity of the platelets. Platelets from a patient with a congenital lack of proaccelerin, a case of parahæmophilia, showed only about 1/50 of the accelerator activity of normal platelets. The main interest has been concentrated upon platelet factor III, which, as found by van Crevald and Paulssen[59], reacts with the antihæmophilic globulin (VIII). Purified extracts containing this factor can restore normal coagulability in cases of thrombocytopenic purpura[60].

The physiological importance of platelets in hæmostasis is not limited to the thromboplastin formation. Morphological studies[61–63] have shown the importance of the agglutination of platelets for hæmostasis and for clot retraction[64]. The platelets also contain a vasoconstricting factor[61], probably 5-hydroxytryptamine, "serotonin"[65]. A local platelet agglutination and a vasoconstriction certainly create a mechanical hindrance to the loss of blood from smaller blood vessels. On the other hand, the clot retraction, facilitated by a platelet factor, may fill a physiological function in partially restoring the patency of recently thrombosed larger vessels (see Ackroyd[54]).

The *chemical reactions* occurring in the thromboplastin formation are completely unknown. The same applies to the activation of proaccelerin to accelerin and to the formation of convertin. Its properties indicate that it may derive from prothrombin.

The first reaction more closely studied is the transformation of prothrombin to thrombin. A prerequisite for this study has been Seegers' preparation of an electrophoretically well-defined prothrombin[66,67]. Thrombin can, as found by Seegers and colleagues[68], be formed from it

in different ways either by means of thromboplastin, or autocatalytically. In a 25 per cent. citrate solution spontaneous activation takes place. During the activation peptides containing a carbohydrate component are released. A similar proteolytic splitting takes place during the fibrin formation. In an excellent investigation, Bailey and Bettelheim[69] in Cambridge in 1952, showed that under the action of thrombin two peptides, A and B, are split off from fibrinogen, one of them, peptide B, with the phenolic hydroxyl of tyrosine conjugated with sulphuric acid[70,71]. The view until recently held by Eagle[72] and Ferguson (see Astrup[73]) that thrombin acts like a plasma protease has thereby been revived. In fact thrombin also acts on a synthetic substrate, splitting tosylarginine methyl ester. The proteolytic action of thrombin is, anyhow, quite specific, taking only one peptide bond out of 1000 in the fibrinogen molecule.

In spite of this splitting off of some peptides from the fibrinogen it is still too early to fully abandon Wöhlisch's[74] view about the denaturating influence of thrombin upon the fibrinogen during the fibrin formation.

Why Does Blood Remain Fluid in the Veins?

We can offer no explanation for this observation. The speed of flow of the circulating blood is considered to be of importance, because in bedridden persons with a greatly reduced flow thrombosis is common. Early ambulation after operation and childbirth strongly reduces the number of thrombo-embolic complications. However, in some hibernating mammals the speed of the blood flow during hibernation is extremely low. Simultaneously there is a marked increase in the number of mast cells in different organs, which could indicate a possible increased production of heparin[75]. But in the blood of most mammals, in human, horse and ox blood, there is practically no heparin to be found, which does not help us to understand the phenomenon.

There are of course other anticoagulating factors of yet unknown importance. Thus Tocantins[76] presented much evidence for the occurrence of a lipid inhibitor of coagulation (see Seeger[57], p. 85). Nilsson[77,78] has recently studied a heparin-like prothrombin inhibitor in mammalian blood.

Also, we know of some protective mechanisms against thrombosis. The first one is the almost instantaneous disappearance of thrombin from the blood, where its presence during life would be very dangerous. Thrombin is strongly adsorbed to the fibrin clot. There are furthermore in the blood strong antithrombins with an action on thrombin *in vitro* as well as *in vivo*. They have been extensively studied during the last years (Astrup[25], p. 101 and Seegers[57], p. 76). They are plasma proteins, some being activated by heparin, which in the presence of a plasma protein, the heparin co-factor, is a very strong antithrombin.

Fibrinolysis, or the dissolution of newly formed fibrin clots, is another very effective but dangerous protective mechanism. There is in the plasma a large excess of plasminogen, a proenzyme which through a yet unknown mechanism may be transformed into plasmin, a proteolytic enzyme highly active on fibrin and fibrinogen. The enzyme makes the

blood more or less inclottable because of fibrinogen deficiency. An activation of plasminogen to plasmin, causing lethal bleeding, sometimes occurs, e.g. in shocked persons, after pneumectomies or abruptio placentæ. In the last mentioned case it is more common that placental thromboplastin enters the maternal circulation, activates prothrombin in the blood and causes multiple emboli of fibrin in the pulmonary vessels. (Seegers[79] *et al.*). This part of the coagulation mechanism demonstrates the complexity of the system by means of which the blood to the benefit of life remains fluid or clots under changing conditions. There are consequently powerful mechanisms in the blood acting in opposite directions, either causing or preventing coagulation, both of them essential to life. If one of them becomes deficient life will be threatened, either by a bleeding tendency, as in hæmophilia, or by a thrombosis. The main problem, yet unsolved, is how these mechanisms counter-balance each other in the blood.

In conclusion I would like to quote Psalm 139, v. 14:—

"I will praise thee; for I am powerfully and wonderfully made: marvellous are thy works; and that my soul knoweth right well."

I think we can all agree that this is the only thing that we really know.

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