Recent Knowledge in Human Plasma Proteins*

By

H. G. Schwick**

The history of human plasma protein research is connected with the names of several famous scientists. Allow me to mention one of them, who in this year celebrated his 80th birthday. It is my teacher, Prof. Dr. H.E. Schultze, with whom I, happy enough, could spend together 20 years of scientific work (Fig. 1).

His contribution to our present knowledge about human plasma proteins is essential and many proteins from human blood plasma were isolated and characterized for the first time by him. Furthermore, he was one of the first, who drew the attention to the immunological determination of plasma proteins and by developing antibodies and highly purified protein antigens he enabled other laboratories to start with these techniques. A highlight of his scientific work is a book, which he has written with the late Heremans, about the molecular biology of human plasma proteins¹⁾ (Fig. 2).

Still today, more than 13 years after this publication has appeared, the book is an important one for all, who are interested in human plasma proteins.

We have, of course, a steadily and quick increase in knowledge about the chemistry and function of

Fig. 1. Professor H.E. Schultze
(1942~1964).

Fig. 2.

* Lecture to the Society of Electrophoresis at the thirtieth Anniversary Meeting of the Society on October 18, 1979.

** Behringwerke AG, Marburg.

human plasma proteins and therefore it is impossible to give you in my lecture a complete survey of what is new in this field. Since I assume that most of you are more clinically interested in human plasma proteins, I will especially refer to newer results on research about the function of plasma proteins.

Definition of human plasma proteins

Let me start with the definition of human plasma proteins. Some time ago, Putnam²⁾ gave a definition, which I think we should accept (Table 1).

Plasma proteins with such criteria might be components of multi-enzyme systems like the clotting system or the complement system or of other proteins systems or groups, which have the same or similar functions or structural similarities or the same place of synthesis. Such proteins are belonging to the group of immunoglobulins, the lipoproteins or the proteinase inhibitors. Furthermore, there is a numerous amount of proteins in human plasma, which does not belong to such systems or groups and of which we even do not know the function up to now.

This definition of plasma proteins excludes hormones, tissue-derived enzymes, erythrocyte-derived proteins as well as numerous proteins, which transit the plasma under physiological or pathophysiological conditions without having a function. The latter were termed by Putnam as "passenger proteins." Finally, we have to take into consideration that there are more than a hundred genetic variants of plasma proteins.

Table 1. Criteria for classification as a true plasma protein.

1. Presence in the plasma (after the neonatal period)
2. Synthesis in liver or RES system rather than in specialized tissues
3. Primary function (including transport function) mediated in the vascular system rather than in a target organ
4. Protein actively secreted into blood rather than by leakage through tissue damage
5. Protein concentration highest in blood of all body fluids
6. Appreciable half life in plasma rather than just a short transit time
7. Protein may exhibit genetic polymorphism or variant forms but polymorphism not due to tissue of origin, as with some isozymes
8. Protein not derived by catabolic proteolytic cleavage in plasma (as Fab or Fc) but may be proteolytic cleavage product of physiological pathways such as complement or coagulation

Putnam, F.W. : Prog. Clin. Biol. Res., 5 : 1, 1976.

Amount of human plasma proteins

There is a great diversity in the normal mean concentration of human plasma proteins. Here I would like to refer to Putnam²⁾ again, who published this figure (Fig. 3) which we have slightly modified and completed by proteins, which in the meantime were described. In this diagram, the normal mean concentration of 65 plasma proteins is plotted as a function of their electrophoretic mobility. Proteins, which belong to systems or groups are marked in the same colour. As it can be seen from this diagram, there is a wide range in concentration of proteins, even between proteins of the same system. The range is from 4 g per 100 ml from albumin to 5 μ g per 100 ml for IgE-globulin.

Of course, the division of plasma proteins into major components, minor components, trace components and ultra-trace components, is arbitrary, but it shows that most of the present known plasma proteins are trace proteins. This fact becomes even clearer with the next diagram (Fig. 4) which demonstrates that more than 90% of the total protein content of human plasma is made up by a few proteins, all the rest,

Fig. 3. Scatter diagram of human plasma proteins.

which includes more than a hundred different proteins, presents together no more than approximately 10% of the total protein concentration of the human plasma. From this we have to understand the difficulty in purifying trace proteins from human plasma and, of course, we surely do not know today of many ultra-trace proteins in human plasma, which might have important physiological functions. In this connexion, I would like to remind you of the fact that the IgE-globulin, which belongs to the ultra-trace proteins, was discovered only because of a few patients with multiple myeloma, which had a 50,000-fold increase of this protein in their serum.

I think this is the right moment to mention that our present knowledge about the variety and the concentration of human plasma proteins was strongly influenced by the development of the technique of immunoelectrophoresis and several quantitative immunological techniques for the determination of plasma proteins.

Very early in the history of determination of plasma trace proteins it was found that the concentration of a great number of proteins in human plasma is age-dependent.^{3,4)} In new born children and during the first time of growing of children the concentration of several plasma proteins among these components of the complement of the clotting and immunoglobulin system are lower than in adults. This is an important point, which has to be kept in consideration, when in connexion with clinical diag-

Fig. 4. Percentage distribution of the human plasma proteins.

nosis plasma proteins are estimated in new born children. Further investigations have soon shown that in elderly human beings under normal physiological conditions an increase or decrease of plasma trace proteins occur⁵⁻⁷⁾ (Table 2).

Also here, in clinical plasma protein diagnosis, one has to refer to responding age-corrected normal values.

There are in addition some plasma trace proteins, for which a sex-dependent concentration was found⁸⁾ (Table 3).

Table 2. Effect of age on serum protein content.

(Immunological determination in pooled sera ; mg/dl)

Age (Years)	15~25	26~45	46~60	61~91
Albumin	4,000	5,000	4,900	3,800
α_1 -Acid glycoprotein	75	79	90	151
α_1 -Antitrypsin	220	270	300	280
α_1 -Lipoprotein	240	360	400	320
α_2 -Macroglobulin	190	150	200	210
β -Lipoprotein	180	320	330	320
Transferrin	240	310	300	320
IgG	1,100	1,610	1,600	2,320
IgA	160	255	270	325
IgM	190	210	220	230

Haferkamp et al. : Gerontologia, 12 : 30, 1965.

Table 3. Serum proteins which show differences according to sex.

(mean values, mg/dl)

Protein	Males	Females
α_2 -Macroglobulin	240	290
Steroid-binding β -globulin	0.4	0.8
IgM	160	201
HDL ₂	40	90

Plasma protein systems

Let me begin the discussion of plasma protein systems in representing two typical multi-enzyme systems of human plasma, the blood clotting system and the complement system. There are similarities in these two systems. Both involve precursor activation and cascade reaction, which lead to intermediary products or new components. Most of the protein components of both systems were highly purified in the last years and well-characterized⁹⁾ (Table 4).

Among the plasmatic coagulation factors eleven are of protein nature. They are different in their molecular weights, their electrophoretic mobilities and their concentration in human plasma.

Functional coagulation factors can be divided into enzymes, accessory factors, catalysts, substrates and proteinase inhibitors (Table 5). To the latter I will refer separately later.

I do not want to go too far into details of the clotting physiology, but I would like to demonstrate on one example, the activation of prothrombin, how far we are today in our protein chemistry understanding of such reactions (Fig. 5).

Table 4. Blood coagulation factors : nomenclature and properties.

Roman numeral designation	Common name	Molecular weight	Relative electrophoretic mobility	Approx. serum concentration (mg/dl)
Factor I	Fibrinogen	340,000	β	200~450
Factor II	Prothrombin	72,000	α_1	5~10
Factor III	Tissue factor			
Factor IV	Calcium ions			
Factor V	Proaccelerin	250~300,000	β	
Factor VII	Procervitin	56,000	α	ca. 0.1
Factor VIII	Antihemophilic factor	ca. $2 \cdot 10^6$	β	ca. 0.1~1
Factor IX	Christmas factor	57~70,000	α_1	0.1~0.7
Factor X	Stuart factor	60,000	α_1	
Factor XI	Plasma thromboplastin antecedent (PTA)	160~210,000	γ	ca. 0.6
Factor XII	Hageman factor	80,000	β	1.5~4.7
Factor XIII	Fibrin-stabilizing factor	340,000	β	1.0~4.0
	Prekallikrein	80,000~127,000	γ	4~5
	HMW kininogen (high molecular weight kininogen)	120,000	α	6
	Plasminogen	87,000	β	10~15

Table 5. Functional characterization of the blood coagulation factor.

1. Enzymes		
a) Proteinases		
F II	Prothrombin	} Prothrombin complex
F VII	Proconvertin	
F IX	Christmas factor	
F X	Stuart prower factor	
F XI	Plasma thromboplastin antecedent	} Contact factors
F XII	Hageman factor	
	Plasma prekallikrein	
	Plasminogen	Fibrinolytic enzyme
b) Glutaminyltransferase		
F XIII	Fibrin-stabilizing factor	
2. Accessory factors		
F III	Tissue factor	
F V	Proaccelerin	
F VIII	Antihemophilic factor	
	HMW-kininogen	
	CIG (cold insoluble globulin)	
3. Catalysts		
	Phospholipid	
	Ca ²⁺ -ions	
4. Substrate		
F I	Fibrinogen	
5. Proteinase-inhibitors		
AT III	Antithrombin III	
C1 INA	C1-inactivator	
α_2 -AP	α_2 -Antiplasmin (fast acting)	
α_2 -M	α_2 -Macroglobulin	
α_1 -A	α_1 -Antitrypsin	

Fig. 5. Activation of prothrombin by factor Xa catalysed by Ca^{2+} and phospholipids.

This figure from Sutti and Jackson¹⁰⁾ shows the fixation of prothrombin to a phospholipid vesical schematically. It is evident that by this reaction principle also a concentration and activation of the clotting factors in the wound area is achieved. For this biological purpose the calcium combining sites on the amino-terminal part of the prothrombin are necessary, in which they differ from other serine esterases. It can be further seen from this figure that thrombin from prothrombin is generated by two consecutive peptide cleavage steps catalysed by factor Xa. In the absence of factor V activation is advancing slowly, however, in it's presence the activation ratio increases in a range, corresponding to a factor of 1,000. Consequently, factor V has an accessory function, because it combines with prothrombin and phospholipid probably, too, it might bring together the different molecules close enough so that the charge transfer for peptide bond cleavage is possible. Finally. it should be mentioned that the prothrombin activator complex is only formed to facilitate it's activation. Already following the first cleavage of prothrombin by factor Xa the residual molecule is separated from the activation complex. The resulting thrombin is free from γ -carboxyglutamic acid residues and does no more differ in this respect from other serine esterases (Fig. 6)¹¹⁾.

The total mechanism of blood clotting is now easily understandable after we know the properties and principles of molecular interaction of the various clotting factors. Clotting can be triggered by two diffe-

Fig. 6. The intrinsic and extrinsic pathways of thrombin formation.

rent stimuli via two pathways, as it is shown in this figure. One is the contact of blood with foreign surfaces; this reaction-pathway has been named the intrinsic one, because it works without factors outside of the vessels, for the other pathway the term extrinsic system is used, it is triggered by a tissue factor, released after injuries. Though definite enzymatic properties on low molecular weight substrates could not yet be attributed to the tissue factor, it apparently activates factor VII, which activates in sequence factor X. At this point of the reaction cascade the intrinsic and extrinsic pathways meet each other.

The onset of the intrinsic clotting mechanism is characterized by two events: the release of platelet factor 3 and the activation of the enzymes of the contact phase, preferently by contact with negatively charged surfaces, Hageman factor (factor XII) is activated, which activates in sequence PTA (factor XI). Both steps of activation are amplified by high molecular weight kininogen and kallikrein, which again is transformed in its active form by factor XIIa. This amplification mechanism guarantees a complete activation of all factors, starting with the endogenous clotting mechanism.

Calcium ions are a prerequisite for the activation of Christmas factor (factor IX) through factor XI. The clotting enzyme generated (factor IXa) forms a complex with factor VIII in presence of calcium and phospholipides called the factor X activator. The activated Stuart factor (factor Xa) formed under the action of the complex and via limited proteolysis, activates the final proteolytic enzyme of the clotting cascade: thrombin.

The molecular events have already been described in detail. It is thrombin, which then converts fibrinogen to fibrin monomers, which aggregate to fibrin strands. These are cross-linked to a stable wound cover by factor XIII. Wound healing is completed by the immigration of the fibroblasts into the wound area, they start the collagen synthesis as soon as they are immobilized by the fixation in the fibrin network through factor XIII.

At this point I have to mention a new discovered protein, the coldinsoluble globulin or fibronectin.¹²⁾ It is a polymorphic glycoprotein, found in blood and tissue of vertebrates and in cultures of adherent vertebrate cells. There are insoluble and soluble forms of fibronectin. Soluble fibronectin is composed of 2 high-molecular weight subunits, held together by disulphide bonds. The molecular weight of the 12-14 S-component is 440,000 D. Insoluble fibronectin may be covalently cross-linked in larger complexes.

Fibronectin has affinities for collagen, fibrin, heparin and cell surfaces. During the final stage of blood coagulation, plasmasfibronectin is crosslinked to fibrin by factor XIII, but the importance of fibronectin is not only due to its participation in the coagulation system, it may be also play a role in neoplastic processes.

There are antibodies available against most of the clotting factors. Therefore, it is now possible to estimate the clotting factors by functional methods as well as immunological methods (Fig. 7).

This figure shows the concentration of some clotting proteins, determined by the rocket electrophoresis according to Laurell. In clinical diagnosis there are some advantages in having the possibility to estimate a clotting factor functional as well as by immunological methods. So it is possible by factor VIII estimations in both ways, to differentiate the von Willebrand's disease¹³⁾ and by the aid of immunological estimations of factor IX three forms of the haemophilia B can be diagnosed,¹⁴⁾ also for the estimation of antithrombin III and factor XIII immunological methods are of advantage for clinical diagnosis.

Turning over to the complement system, so far 21 plasma proteins are known, which are involved in the different activation steps of the complement reaction.¹⁵⁻¹⁷⁾ All of them could be purified and physico-chemically characterized (Table 6).

Similar to the components of the blood coagulation systems, the complement proteins are different in their molecular weight and electrophoretic mobility. Also their concentration in human plasma varies greatly. Most of the complement factors belong to the minor or trace proteins (Table 7, 8).

Fig. 7. Immunological determination of six clotting factors.
The first four wells of each plate were filled with plasma dilutions to set up a calibration curve.
AT III=Antithrombin III, PP=Plasminogen.

Table 6. Early acting proteins of the classical pathway of human complement system.

Component	Molecular weights of		Approx. serum concentration (mg/dl)	Relative electrophoretic mobility
	Intact molecule	Chains*		
C 1 q	410,000	18 { 6A 24,000 6B 23,000 6C 22,000	15	γ_2
C 1 r	83,000**	1	5	β
C 1 s	83,000	1	5	α
C 2	110,000	1	1.5	β_1
C 3	180,000	2 { α 105,000 β 75,000	120	β_2
C 4	206,000	3 { α 93,000 β 78,000 γ 33,000	40	β_1

* Chains obtained after reduction and alkylation of disulphide bonds.
** C 1 r is present as a dimer of two identical noncovalently linked chains in serum.

Table 7. Proteins of the alternative pathway of human complement system.

Component	Molecular weights of		Approx. serum concentration (mg/dl)	Relative electrophoretic mobility
	Intact molecule	Chains*		
C 3	180,000	2 { α 105,000 β 75,000	120	β_2
B (C 3 Proactivator)	93,000	1	20	β_1
D (C 3 Proactivator convertase)	24,000	1	0.25	α
P (Properdin)	184,000	4** 46,000	2	γ
C 3 b INA	93,000	2 { α 55,000 β 42,000	3.4	β
β_1 -H	150,000	1	50	β

* Chains obtained after reduction and alkylation of disulphide bonds.
** Properdin is composed of four similar, noncovalently linked subunits.

Table 8. Proteins of the terminal pathway of human complement system.

Component	Molecular weights of		Approx. serum concentration (mg/dl)	Relative electrophoretic mobility
	Intact molecule	Chains*		
C5	180,000	2 { α 105,000 β 75,000	8	β_1
C6	128,000	1	7.5	β_2
C7	121,000	1	5.5	β_2
C8	174,000	3 { α 77,000 β 63,000 γ 13,000	8	γ_1
C9	79,000	—	23	α

* Chains obtained after reduction and alkylation of disulphid bonds.

The two pathways of activation of complement, the classical and the alternative one, are schematically shown in this figure of Müller-Eberhard,¹⁵ who has together with his group remarkably contributed to our present knowledge of complement proteins (Fig. 8).

Again, I do not want to go too far into the mechanism of complement activation. This could be the content of several lectures.

There are antibodies available against the most of the complement proteins. For clinical diagnosis it is mostly sufficient to estimate the complement factors C2, C3, C4 and factor B. This factors can be, in different extent, decreased in plasma of patients with severe infections. Furthermore, in patients with SLE-like symptoms and in immune complex diseases, in which the early factor of the classical pathway, C2 and C4, are consumed and the decrease of factor B in this case reflected the activation of the alternative pathway.¹⁸⁾

Closely related to these two systems of coagulation and complement is a group of plasma proteins with antiproteinase activity. They interact in the different cascade activation steps and are responsible for the inactivation of activated enzymes. The special importance of this proteinase inhibitors is that they are essential for the balance of not only one multi-enzyme system. That means, certain proteinase inhibitors are with a given priority acting in two or more multi-enzyme systems (Table 9).

So far, we have knowledge of eight proteinase inhibitors in human plasma.¹⁹⁾ They were highly puri-

Fig. 8. Pictorial representation of the orderly reaction sequence of complement.

fied and well characterized. In comparison to the other plasma proteins I mentioned, the concentration of proteinase inhibitors in human plasma is relatively high. They mostly belong to the major or minor proteins in the terminology of Putnam (Table 10).

Table 9. Concentration and characterization of the inhibitors in human serum.

Inhibitors	Molecular weight	Conc. mean±SD (mg/dl)	Relative electrophoretic mobility	Carbohydrate content (%)
1. α_1 -Antitrypsin	54,000	290.0±45.0	α_1	12.2
2. α_1 -Antichymotrypsin	69,000	48.7± 6.5	α_1	24.6
3. Inter- α -Trypsininhibitor	160,000	50.0	$\alpha_1\sim\alpha_2$	8.4
4. Antithrombin III	65,000	23.5± 2.0	$\alpha_1\sim\alpha_2$	13.4
5. C 1-Inactivator	104,000	23.5± 3.0	α_2	34.7
6. α_2 -Macroglobulin	725,000	260.0±70.0	α_2	7.7
7. α_2 -Antiplasmin (fast acting)	70,000	7.0	α_2	14.0
8. Inhibitors of the plasminogen activation				

Table 10. Spectrum of action of the plasmatic proteinases inhibitors.

Name	Proteinases							
	Function	α_1 -A	α_1 -X	I α I	AT III	C1 INA	α_2 -M	α_2 -AP
Thrombin F IIa	Coagulation	—	—	—	+	—	+	+
Clotting F Xa		—	—	—	+	—	—	—
Clotting F XIa		—	—	—	weak	+	—	+
Clotting F XIIa		—	—	—	weak	+	—	+
Plasmin	Fibrinolysis	+	—	—	weak	+	+	+
C 1 r	Complement	—	—	?	—	+	—	—
C 1 s		—	—	?	—	+	—	—
Prekallikrein-activator	Kallikrein			—	—	+	—	?
Plasma kallikrein		+	—	—	weak	+	+	+
PF/Dil	Permeability	—	—	?	—	+	?	?
Acrosin	Fertilisation	+	—	+	+	—	+	?
Trypsin	Pancreat. Hydrolys.	+	—	+	+	weak	+	+
Chymotrypsin		+	+	weak	—	weak	+	—
Elastase	Phagocytosis	+	—	—	—	—	+	?
Collagenase		+	—	—	—	—	+	?
Cathepsin D		—	—	—	?	?	+	?
Cathepsin G		?	+	?	?	?	?	?
Papain	Metabolism	—	—	?	?	?	+	?
Bromelin		—	—	?	?	?	+	?
Ficin		—	—	?	?	?	+	?

+ =strong, probably stoichiometric inhibition

? =not determined

Heimburger, N. : In Proteases and Biological Control (E. Reich, D. Rifkin, E. Shaw, eds.) Cold Spring Harbor Laboratory. Cold Spring Harbor N. Y. 1975.

From this table one can see that practically all of the proteinase inhibitors are capable to neutralize more than one proteinase. Furthermore, it can be seen what I mentioned before, namely that some of the inhibitors are involved in more than one multi-enzyme system. (Table 11)

Here is what we know so far about the physiological function of the proteinase inhibitors. For clinical diagnosis the proteinase inhibitors can be estimated functionally as well as by immunological methods.

In addition to what is shown in this table about the function of the most important proteinase inhibitors of the human plasma I would like to mention some newer data about a few of these proteinase inhibitors. α_2 -macroglobulin is of great importance, because it forms complexes with urokinase as well as with trypsin, chymotrypsin, plasmin and thrombin, furthermore it was found that it is a zinc-binding globulin, it binds two atoms of zinc per mol α_2 -macroglobulin.²⁰⁻²²⁾ Recently it was found that the α_1 -antichymotrypsin is an acute phase protein, the plasma concentration of which may double within eight hours after tissue damage.²³⁾ So it is believed that it's primary role is the regulation of chymotrypsin-like enzymes, particularly for those enzymes released during an inflammatory episode. Moreover, it was found that an α -protein, which was detected in perchlorid acid extract of malign tumors, normal tissues, sera and other body fluids, is identical with the α_1 -antichymotrypsin.²⁴⁾ It is also believed that one of the primary functions of α_1 -antichymotrypsin is to inactivate leucocyte cathepsin G.²²⁾

In the case of inter- α -trypsin inhibitor it was found that 30 to 60% of patients with disseminated neoplastic diseases excrete in their urine 100 to 500 mg per day of a glycoprotein with a molecular weight of 27,000, which is antigenically and chemically related to the inter- α -trypsin inhibitor and may be a cleavage product of the latter.²⁵⁾

Recently Aoki and others²⁶⁾ reported on a congenital deficiency of α_2 -plasmin inhibitor in a 25 year old Japanese man, associated with severe hemorrhagic tendency. It is assumed that the α_2 -plasmin inhibitor deficiency has led to uninhibited in vivo fibrinolysis, which probably causes the severe hemorrhagic

Table 11. The physiological function of the most important inhibitors.

Inhibitor	Function	Pathology
C1 INA	Activation control of : blood coagulation, fibrinolysis, kallikrein, complement	Hereditary angioneurotic edema : swelling of skin and mucous membranes due to a decreased or functional inactive inhibitor.
AT III, α_2 -M	Local limitation of coagulation	Hereditary AT III deficiency : thromboembolic disorders. Consumption of AT III : Hypercoagulability.
α_2 -M, C1 INA, α_1 -A α_2 -M, α_1 -A, Ia-I	Limitation of fibrinolysis to the pathological substrates. Control of infections and inflam- mations on the level of proteinases involved, their neutralization and clearance. Prevention of autodi- gestive processes and side reac- tions to the coagulation system.	consumption of α_2 -M during fibrinolytic therapy. Hereditary α_1 -A deficiency : pulmonary emphysema. Local consumption of inhibitors during : acute inflammations, infections of the nasal mucous membrane, rheumatoid ar- thritis in the synovial fluid, in burned patients, leukemia, endotoxin shock.

Heimburger, N. : In Proteases and Biological Control (E. Reich, D. Rifkin, E. Shaw, eds.) Cold Spring Harbor Laboratory. Cold Spring Harbor N. Y. 1975.

tendency.

There is no doubt that with the proteinase inhibitors in human plasma we have a protein system, which consists of proteins with similar functions but at that time we do not know so much about a similar chemistry of these proteinase inhibitors. By amino acid analysis there was found a strong correlation in sequence homology between α_1 -antitrypsin and α_1 -antichymotrypsin, between the residues 16 to 27 of α_1 -antichymotrypsin and the residues 33 to 44 of α_1 -antitrypsin.²⁷⁾ Moreover, it was established that the unusual terminal arginine in α_1 -antichymotrypsin is also shared by the inter- α -trypsin inhibitor, but until more sequence data are available, the possible relationship in the structure of all the plasma proteinase inhibitors cannot be considered.

In another protein system of human plasma, I would like to mention an example, in which structural homology and functional similarities as well as the places of synthesis are shared by all members of this system. It is the immunoglobulin system, of which, due to an enormous scientific work, which was going on in the last 20 years, we all know more than about other plasma proteins²⁸⁾ (Fig. 9).

Fig. 9. Relative concentration of human immunoglobulins.

Table 12. Properties of human immunoglobulins.

Ig	Chain structure	Mol. wt. (x 10 ⁻³)	Serum conc. (mg/dl)	Half-life (days)	Fixation of complement	Attachm. to monocytes
IgG1		146	900	21±5	+	+
IgG2		146	300	20±2	+	-
IgG3		165	100	7±1	+	+
IgG4		146	50	21±2.5	a.p.	-
IgM		970	150	5	+	-
IgA1		160	300	6	a.p.	-
A ₂ m(1)						
IgA2						
A ₂ m(2)		160	50	6	a.p.	-
sIgA		380-390	0.5		-	
IgD		172-200	3	3	-	
IgE		168-198	0.03	2.3-4	a.p.	

Therefore, I think it is not necessary to comment on immunoglobulins in my lecture too much. This picture should remind you of the great differences in the concentration of the five immunoglobulins and in the next table I have summarized the physicochemical data (Table 12). I should mention again here, which tremendous contribution immunological methods have to our today's knowledge of immunoglobulins and immunoglobulin structure. Especially the discovery of the technique of immunoelectrophoresis by Grabar was an essential step into this research.

Transport proteins of human plasma

It is not possible to put together all proteins of human plasma, which have the properties of transport proteins to a protein system like I have given examples before. There are too many differences in the physicochemical behaviour, also regarding the substances that have to bind or to transport and the way how they do it. Furthermore, they have different synthesis places. In spite of all this, I will try to discuss the transport proteins of human plasma together (Fig. 10).

Many of the today's known plasma proteins have transport functions, but albumin has of course the pre-dominant transport function of all plasma proteins. It is impossible to mention all substances of endogenous or exogenous nature, which are transported by albumin. With this figure I would like to remind you that the technique of electrophoresis has been of great importance for the detection of transport proteins, especially of the function of albumin in this respect. Simply by adding substances in the Tiselius electrophoresis to serum, the transport function of plasma proteins has been demonstrated in the beginning of this research area. This work is closely connected with the name of Prof. Bennhold in Germany (Table 13).

Great advances were made in structural characterization of transport proteins and their binding sites. Last year it was reported that bilirubin is bound to the lysine residue 240 at it's high affinity site on human serum albumin.²⁹⁾

Prealbumin is a plasma protein that is of considerable biological importance because it transports both, a hormone and a vitamin in serum. Thyroxine is directly bound to the prealbumin molecule, while retinol (vitamin A alcohol) is indirectly bound through a protein-protein interaction involving it's specific carrier molecule, retinol-binding protein^{30,31)} (Fig. 11).

In cooperation with our group, scientists of the Crystallographic Institute in Paris and the Laboratory of Molecular Biophysics in Oxford succeeded to investigate the structure of prealbumin in a 2.5 Å resolu-

Fig. 10. Serum protein components responsible for the blood transport of anions, cations, dyes, and drugs.

Table 13. Transport proteins of human plasma.

Protein	Biological function	Molecular weight	Normal concentration (mg/dl)
Prealbumin	Transport of thyroxine and retinol binding protein	54,980	25
Albumin	Transport of ions, pigments, etc. osmotic function	66,000	3,500~5,500
Transcortin	Binding and transport of cortisol	55,700	4
Thyroxine binding globulin	Thyroxine binding	54,000	1~2
Retinol binding protein	Binding and transport of retinol (vitamin A)	21,000	4.5
Gc-globulin	Binding and transport of vitamin D ₃	50,000	40
Transcobalamin I	Binding and transport of vitamin B ₁₂	60,000	?
Transcobalamin II	Binding and transport of vitamin B ₁₂	53,900	traces
Ceruloplasmin	Copper binding ; oxidase	132,000	35
Transferrin	Iron binding and transport	76,500	295
Hemopexin	Hemin binding	57,000	80
Haptoglobin	Hemoglobin binding	100,000 (Typ 1-1)	170~235
α_1 -Lipoprotein (HDL)	Transport of lipids, hormones etc.		360
β -Lipoprotein (LDL)	Transport of lipids (cholesterol, hormones etc.)		400

Fig. 11.

tion.^{32,33)} The two binding sites for thyroxine are in a channel, which runs through the center of the molecule and is formed by the tetrahedral arrangement of four identical subunits. The complete prealbumin retinol-binding complex can carry one thyroxine molecule and four retinol molecules. By affinity labeling of the thyroxine-binding domain of human serum prealbumin with dansylchloride it was shown by Cheng and others,³⁴⁾ after various fractionation procedures that the labeled residue is lysine 15. This lysine, which is located in the central channel formed by the four subunits of prealbumin is therefore within the dansyl and thyroxine-binding domain of prealbumin.

Also about thyroxine-binding globulin we have some newer data concerning its structure.³⁵⁾ From the result so far it can be concluded that this protein is a single polypeptide chain, the secondary and tertiary structure of human plasma thyroxine-binding globulin was investigated by circular dichroism and fluorescence properties. The relaxation time of TBG indicated that it is a compact symmetric molecule, of which about one half of the peptide groups are equally distributed in α -helical and β -structures.

It was very interesting, when about four years ago it was found that the group-specific component of human plasma, the so-called Gc-globulin, is identical with transcalsiferin, a transprotein of human plasma, which is a vehicle for vitamin D. In the meantime we know that this protein has a single binding site for 25 hydroxyvitamin D₃ and is probably also responsible for the transport of other vitamin D metabolites, present in serum.³⁷⁾ It has a high affinity and due to its large serum concentration also a high capacity for 25 hydroxyvitamin D₃ and therefore it probably represents a storage protein, protecting the organism from certain vitamin D deficiency or toxication (Fig. 12).

This figure shows an immunoautoradiography of an electrophoresis demonstrating the vitamin D-binding of Gc-globulin.

Our knowledge about the transcobalamin I and II regarding structural composition is not so great. This vitamin B₁₂-binding proteins present in normal human serum, can be separated chromatographically into two main fractions, which are termed transcobalamin I and II. Transcobalamin I is a glycoprotein with about 33% carbohydrate, whereas transcobalamin II is no glycoprotein.^{38, 39)}

From haemopexin, a haem-binding serum protein, which was first described 20 years ago,⁴⁰⁾ we know today that it is a protein, which consists of a single polypeptide chain and contains about 20% of carbohydrate. It binds equimolar amounts of porphyrins and metallo-porphyrins.^{41, 42)}

Haptoglobin, the haemoglobin-binding protein in serum, exhibits genetic polymorphism in human population.^{43, 44)} Haptoglobin from homozygous haptoglobin 1-1 individuals is electrophoretically a single molecular species. Haptoglobin from both, homozygous haptoglobin 2-2 and heterozygous haptoglobin 2-1 individuals each consist of a series of polymeric proteins of increasing molecular weight, with nine to

Fig. 12. Identification of Gc-globulin as the vitamin D-binding protein.

Immuno-autoradiography of individual human serum samples labeled with [¹⁴C] vitamin D₃ and tested against antiserum to human Gc-globulin.

Daiger et al. : Proc. Nat. Acad. Sci. U.S.A., **72** : 2076, 1975.

twelve easily demonstrable proteins for haptoglobin 2-2 and a different series of more than seven for haptoglobin 2-1. It was found that all haptoglobins were composed of two unequal polypeptide chains.⁴⁵⁾ The smaller unit was designated the α -chain, the larger the β -subunit is common to these haptoglobin types.

From the well-known metallo-proteins transferrin and ceruloplasmin only transferrin is a true transport protein. Ceruloplasmin, as we know for a long time, is an enzyme and regarding to its structure it should be mentioned that there is chemical evidence that ceruloplasmin is a single chain protein and that the so-called subunits, which are reported in earlier publications, are fragments due to proteolytic cleavage of the ceruloplasmin⁴⁶⁾.

To complete the story about transport proteins, I have to mention the plasma lipoproteins. The study of plasma lipoproteins in recent years has significantly contributed to our understanding of lipid metabolism and lipid disorders.⁴⁷⁻⁴⁹⁾ The lipoproteins are complex macromolecules containing lipid non-covalently bound to protein and carbohydrate. They are generally separated into four families according to physical characteristics and chemical composition. It is not possible to go further into the action and composition of lipoproteins, but I might refer to very good reviews about this topic (Table 14).

What is new in clinical diagnostic with transport proteins? In a study, where plasma concentration of Gc-globulin (vitamin D-binding protein) has been measured in healthy subjects, pregnancy and during oestrogen therapy neither sex- nor age influenced the plasma vitamin D-binding globulin concentration in healthy subjects, but there was a significant increase in concentration during pregnancy and oestrogen therapy. Elevated levels were found in vitamin D-deficient elderly but not younger subjects, while levels in subjects with chronic liver disease were significantly reduced.⁵⁰⁾

Regarding thyroxine-binding globulin I would like to mention two findings, which are of clinical importance. It was found that TBG does not follow the progressive degrees of T₄ and T₃ with age.⁵¹⁾ Thus age-dependent euthyroid ranges for thyroid hormone concentration including TBG-concentration should be established for better clinical discrimination of thyroid function.

The other finding is that in the acute phase of kwashiorkor, TBG maximal binding capacity of thyroxine is significantly decreased (56.1% of normal). TBG maximal binding capacity returns to normal after two weeks of nutritional treatment.⁵²⁾

For prealbumin it was recently found that it has thymosin-like activity.⁹⁸⁾ This would be a very interesting finding, if it can be confirmed by other groups.

Table 14. Distribution of plasma apolipoproteins.

(% of total lipoprotein)

Apoprotein	Chylomicrons	VLDL	LDL	HDL
A ₁	?	T>	—	65~70
A ₂	?	?	—	20~25
B	5~20 ?	40+	>95	T>
C ₁	15*	10+	T>	1~3
C ₂	15*	10+	T>	1~3
C ₃	15*	30+	T>	5~10

* Estimate.

+ Varies with VLDL subspecies, denser VLDL has more B relative to C.

Levy, R.L.: Proc. Clin. Biol. Res., 5: 25, 1976.

Human plasma proteins with unknown function

More than ten α - and β -glycoproteins of human plasma have been identified and physicochemically characterized, for which so far no function is known. Most of them were isolated and for the first time described by our group of the Behring Institute. Let me go briefly through what we meanwhile have learnt about some of these proteins (Table 15).

The α_1 -acid glycoprotein, which has a relatively high concentration in human plasma, is owing to the work of K. Schmid and his colleagues well-known in its structure^{53,54} (Fig. 13).

It consists of one polypeptide chain with two disulphide bonds and five carbohydrate units. It is very interesting that the amino-acid sequence of α_1 -acid glycoprotein possesses significant similarity with the immunoglobulins. Therefore, it is discussed that α_1 -acid glycoprotein is probably related to the immunoglobulins and that it possibly is diverged from the immunoglobulin evolutionarily, prior to the formation of the primitive L-chain.

Table 15. Human plasma proteins of unknown function.

Protein	Molecular weight	Carbohydrate content (%)	I. P.	Concentration mg/dl plasma
α_1 -Acid glycoprotein	41,000	73.9	2.7	90
α_1 -T-glycoprotein	60,000	13.7		8
α_1 -B-glycoprotein	50,000	13.3	4.4~4.6	22
9.5S- α_1 -glycoprotein	308,000	12.8	5.0	5.5
α_1 -Microglobulin	26,700	20		5.4
Zn- α_2 -glycoprotein	41,000	18.2	3.8	5
α_2 -HS-glycoprotein	49,000	13.4	4.1	60
Histidine-rich 3.8S- α_2 -glycoprotein	58,500	14.3	5.6~6.2	9
Leucine-rich 3.1S- α_2 -glycoprotein	49,600	23	3.8~4.1	2.1
8S- α_3 -glycoprotein	220,000	31.4	4.2~4.6	4
4S- α_2/β_1 -glycoprotein	60,000	27.7	4.0	0.02
β_2 -Microglobulin	11,800	0	5.56	0.15
β_2 -Glycoprotein I	40,000	18.8	5.4~6.25	20
β_2 -Glycoprotein III	35,000	10	5.1~5.8	10
C-reactive protein	110~140,000	0		<0.1

Fig. 13. Schematic presentation of α_1 -acid glycoprotein.

In this respect it is further of interest that it was found that α_1 -acid glycoprotein with a molecular weight of 52,000 is present in the membrane of normal human lymphocytes, granulocytes and monocytes. It is synthesised by lymphocytes and apparently subsequently cleaved as a soluble serum form with a molecular weight of 41,000.⁵⁵⁾ α_1 -acid glycoprotein belongs to the acute phase proteins and elevated serum concentrations of α_1 -acid glycoprotein under various conditions like inflammation, major surgery and cancer, which are also associated with proliferation of leucocytes, can be at least in part explained as originated from such cells.

Finally, I would like to mention that it has been shown that α_1 -acid glycoprotein binds different steroids, basic drugs like alprenolol and imipramine⁵⁶⁾ and that this protein markedly inhibits platelet aggregation⁵⁷⁾.

Whereas we have no new data about α_1 -T⁵⁸⁾ and α_1 -B-glycoprotein,⁵⁹⁾ in the case of 9.5 S- α_1 -glycoprotein,⁶⁰⁾ which has a high affinity to polyanions, we know today that this protein is identical with the P-component of human plasma, which could be identified by electronmicroscopy in amyloid laden organs with a pentagonal structure.⁶¹⁾ Later it was found that the so-called C1t-subcomponent of human complement, described by Painter,⁶²⁾ is also identical with 9.5 S- α_1 -glycoprotein.⁶³⁾

There is nothing new with the Zn- α_2 -glycoprotein,⁶⁴⁾ but about α_2 -HS-glycoprotein⁶⁵⁾ it was reported that it is a constituent of both foetal and adult human bone matrix and is incorporated and concentrated into bone presumably through interactions with one of the other components of mineralizing matrix.⁶⁶⁾ It was also found that α_2 -HS-glycoprotein possesses obsonic properties and that it is markedly decreased after various surgery procedures.

Also about histidine-rich 3.8 S- α_2 -glycoprotein⁶⁷⁾ there are some new data. This protein is composed of two non-identical subunits, each of which contains carbohydrates. The far ultraviolet circular dichroism spectrum of α_2 -histidine-rich glycoprotein indicates that the protein has little α -helix but apparently appreciable amounts of β - and non-regular structures.⁶⁸⁾

Histidine-rich 3.8 S- α -glycoprotein has been found to bind heme but it does not effectively compete with human serum albumin for heme, suggesting that α_2 -histidine-rich glycoprotein has no major function in serum-heme-transport. Furthermore, it has been shown by in vitro experiments that the protein is able to bind divalent zinc and copper.⁶⁹⁾

First studies about the role of histidine-rich 3.8 S- α_2 -glycoprotein and the clinical applications of monitoring levels of this protein in disease stages have shown a decline of the protein in pregnancy, elevated levels in patients with a variety of heart failures including myocardial infarction, rheumatic heart disease and heart valve surgery, also in patients with erythropoietic protoporphyria high levels were found.⁶⁹⁾

As far as we know, there are no new data about 8 S- α_3 -glycoprotein⁷⁰⁾ and 4 S- α_2 - β_1 -glycoprotein.⁷¹⁾ Also about the leucine-rich 3.1 S- α_2 -glycoprotein, which we discovered two years ago,⁷²⁾ we do not know it's physiological function. This protein has a remarkably high amount of leucine (17%), which means that every fifth amino-acid is leucine.

α_1 -microglobulin is a glycoprotein, which was first isolated by Swedish groups^{73,74)} from urine of patients with tubular proteinuria, it is present in serum and urine of healthy individuals as well. In your country, Takashi and others⁷⁵⁾ have recently shown the tissue distribution of human α_1 -microglobulin. They could localized the protein on the lymphocyte surface both on T- and B-lymphocytes. From this it is discussed that lymphocytes are the primary source of α_1 -microglobulin. Clinically this protein is found at elevated levels in the serum and urine of patients with renal failures. It remains open, whether the α_1 -micro-glycoprotein, which was isolated by Seon and Pressmann⁷⁶⁾ from the urine of a cancer patient, is identical with the α_1 -microglobulin.

Since the discovery of β_2 -microglobulin about ten years ago by Berggard and Bearn⁷⁷⁾ many studies

were undertaken with this protein, the results of which are well-known. I only would like to remind you of its structural and evolutionary relationship to immunoglobulins, the identification of β_2 -microglobulin as a membrane protein and the discovery of the association of β_2 -microglobulin with histocompatibility antigens.⁷⁸⁾ To this I only want to add one clinical finding. From the urine of a patient with viral hepatitis and a bleeding disorder a protein could be isolated, which interferes with the fibrin polymerisation. From immunological analysis and the molecular weight of this protein, this inhibitor seems to be identical with the β_2 -microglobulin.⁷⁹⁾

It's now eighteen years ago, when we described for the first time the β_2 -glycoprotein I, a carbohydrate-rich protein, which we were able to crystallise.⁸⁰⁾ Later we found a family, in which this protein was absent in serum without leading to any disease.⁸¹⁾ The physiological nature of this protein is still unknown (Fig. 14).

Burstein has shown that in presence of β_2 -glycoprotein I triglyceriderich lipoproteins were precipitated by synthetic anionic detergents⁸²⁾.

We could show that β_2 -glycoprotein I directly precipitates with dextran sulphate and heparin (Fig. 15). Maybe we will learn more about the nature of this protein, because Putnam's laboratory is just under taking structural studies of β_2 -glycoprotein I.

It is also a long time ago when we described the β_2 -glycoprotein III for the first time.⁸³⁾ In the meantime there was one report, which gives a little light to the physiological behaviour of this protein. It has been shown by Chase and Prochaska that β_2 -glycoprotein III is normally present in kidneys.⁸⁴⁾

Fig. 14. Genetic deficiency of β_2 -glycoprotein I.

Fig. 15. Precipitin reaction of β_2 -glycoprotein I with heparin.

In many diseases, in which is immunohistochemical or ultrastructural evidence of immune complex deposits, in glomeruli or peritubular tissue there are strong immunofluorescence staining reactions for β_2 -glycoprotein III, suggesting that this serum glycoprotein may be associated with immune complexes

The C-reactive protein is the prototypic acute phase reactant. Since the beginning of this year we know it's primary structure.⁸⁵⁾

The protein yielded a unique sequence containing 187 amino-acids in a single polypeptide-chain. It consists of a single kind of subunit with a molecular weight of approximately 21,500. In serum as well as in purified state CRP has a variable molecular weight ranging from 110,000 to 144,000. Electron-microscopic studies revealed that the subunits of CRP aggregate preferentially as cyclic pentamers and that this may state to form decamers.⁸⁶⁾ Here we have a similarity to the 9.5 S- α_1 -glycoprotein or P-component, which indeed shows also homology in amino-acid sequence with CRP.⁸⁶⁾

Since CRP and immunoglobulins have a similar amino-acid composition, it was assumed that CRP might share a common evolutionary origin with immunoglobulins.⁸⁷⁾ From the newer finding about the primary structure of CRP this hypothesis can be excluded.

The physiological function of CRP remains still unknown, also there are in the past several data about biological activities of CRP such as promoting reaction of precipitation, of agglutination, capsular swelling, phagocytosis, complement fixation and binding to antigen-induced but not mitogen-induced T-lymphoblasts.^{88~90)} Furthermore, it has been shown that CRP inhibits platelet aggregation.⁹¹⁾ Therefore, it is assumed that CRP serves to modulate platelet reactivities during acute inflammatory reactions.

Let me finish this chapter of human plasma proteins with unknown function with the demand to investigate this proteins in several diseases to learn more about the physiological function.

The function of carbohydrates in human plasma proteins

Most of the human plasma proteins are glycoproteins, only a few including albumin are free of carbohydrates (Fig. 16).

Therefore, I think I should refer in this lecture shortly to the function of carbohydrates in glycoproteins as far as our understanding is today.⁹²⁾

The chemistry of the carbohydrates in human glycoproteins is following certain rules : only asparagin, threonine or serine can be carrier of the oligosaccharide groups and in the case of the N-acetyl-glucosamine bounds by participation of asparagin, the peptide-chain is characterized by the groups ASN-X-THR or ASN-X-SER. The carbohydrates, which are found in glycoproteins, are N-acetyl-glucosamine, N-acetyl-galactosamine, galactose, manose, glucose, fucose, N-acetyl-neuraminicacid (sialic acid) and xylose.

Among the different functions of the carbohydrate moieties in glycoproteins, which were discussed,

Fig. 16. Carbohydrate-free human serum proteins.

Table 16. Function of the carbohydrate moieties of glycoproteins.

Secretion-mechanism, Eylar's hypothesis
(Transport through cell membranes, placenta)
Glycosyltransferases Asn-X-Thr/Ser coded
Increasing of solubility
Resistance against proteolytic enzymes
Vestiges of the evolutionary process
Recognition-mechanism

most scientists agree today that the recognition mechanism is the most important one. It is believed that the carbohydrates in glycoprotein have therefore important functions for cell-interaction, adhesion and recognition. The rapid metabolism of sialic acid-free glycoprotein like ceruloplasmin is an example for a positive recognition. As long as the terminal sialic acid is bound to ceruloplasmin, the parenchymal cells of the liver do not recognize the protein (Table 16).

After cleavage of the sialic acid, the free residues of galactose were recognized immediately, probably through sialyl-transferase of the cell-membrane, and the asialo-ceruloplasmin is eliminated very quickly from the blood.

Negative recognition for example is the masking or covering of antigenic determinants or of greater areas of the surface of molecules, here for example serum glycoproteins as inhibitors of haemagglutination by influenza virus (Table 17).⁹³⁾

Table 17. Serum glycoproteins as inhibitors of hemagglutination by influenza virus (strain B/Md)^{a)}.

Compound	Inhibitor activity ^{b)}	Molecular weight	Neuraminic acid	
			(%)	(mole/mole)
Blood group substance MM	100	12,000,000	11.5	4,466
Uromucoid ^{c)}	400	7,000,000	7.0	1,586
IgM globulin	25	900,000	1.8	405
Haptoglobin 1-1	25	85,000	5.2	14
Fetuin (bovine)	12.5	45,000	4.5	9
Hemopexin	10	80,000	5.0	13
α_1 -X-Glycoprotein	6.25	60,000	7.0	14
α_2 -Macroglobulin	5	820,000	1.8	48
β_2 -Glycoprotein	1.67	40,000	4.5	6
α_1 -Acid glycoprotein	0.80	44,000	12.1	17
Ceruloplasmin	0.80	160,000	2.4	12
Zn- α_2 -Glycoprotein	0.80	41,000	4.7	6
IgA globulin	0.80	160,000	1.3	11
α_1 -Antitrypsin	0.53	54,000	3.6	6
α_1 -T-Glycoprotein	0.40	60,000	7.0	7

a) Springer, G.F., Schwick, H.G. and Fletcher, M.A. : Proc. Natl. Acad. Sci. U.S.A. **64** : 634-641, 1969.

b) Percent of blood group substance MM on weight basis.

c) Tamm, I., and Horsfall, Jr., : Proc. Soc. Exp. Biol. Med., **74** : 108-114, 1950.

For the characterization and estimation of glycoproteins, lectins are of great importance. In a broader sense lectins can be regarded as anticarbohydrate reagents of non-antibody-like nature. They occur in plants, invertebrates and vertebrates and their behaviour as carbohydrate recognition molecules can be exploited as a tool and a basis for the study of their biological function.

Recently there was given evidence for lectin properties of human plasma proteins.⁹⁴⁾ Together with Uhlenbruck, as a curiosity we first observed a completely unexpected precipitin reaction between asialo ceruloplasmin and the 9.5 S- α_1 -glycoprotein (Fig. 17)⁹⁵⁾.

This finding prompted us to test other serum proteins and other galactans for their ability to precipitate with the 9.5 S- α_1 -glycoprotein.⁹⁶⁾ However, whereas the asialo-ceruloplasmin remained the only serum glycoprotein being positive in this respect, galactans from different sources, mainly of snail origin, did react (Fig. 18).

Fig. 17. Precipitin reaction of the rutilus lectin with certain serum asialoglycoproteins.
The reaction between asialoceruloplasmin and asialo-9, 5 S- α_1 -glycoprotein.

Glycosubstance (origin and nature)	CRP ^{a, b)}	Serum protein C1q ^{b)}	9.5S α_1 -glyco- protein ^{a)}
Helix pomatia snail galactan	+	+	+
Cepea nemoralis snail galactan	+	+	+
Helix aspersa snail galactan	+	+	+
Helix lucorum snail galactan	+	-	+
Bovine pneumo galactan	-	+	+
Littorina littorea snail galactan	+	+	+
Borus snail galactan	+	-	-
Pomacea urceus snail galactan	-	-	+
Pneumococcus type XIV polysaccharide	+	-	-
Porcine peptone A glycoprotein (gastric juice)	-	+	-
Pig amnion mucoid	-	+	-
Pig erythrocyte glycoprotein	-	+	-

a) Buffer: Tris-GaCl₂, pH 8.5.

b) Barbitol-bul. r 0.05 M, pH 8.5.

Fig. 18. Precipitin reactions of CRP, C1q and 9.5S α_1 -glycoproteins with certain glycosubstances.

The human serum proteins, the reactive protein, the 9.5S- α_1 -glycoprotein and the complement factor C1q show, when tested in agar-gel diffusion under certain buffer conditions, strong precipitin reactions with polysaccharides of the galactan type, indicating that they may have lectin-like recognition sites. Further studies have to be undertaken to investigate the possible physiological function of this lectin-like plasma proteins.

Genetic alteration in human plasma proteins

In a lecture about recent knowledge in human plasma proteins I have to include the genetic alterations of human plasma proteins at least very comprehensively.

All the plasma proteins are subject to genetic control. The genes, which are responsible for the synthesis of these proteins, have mutated in the course of evolution and so we have considerable deviations in the structure and activity of plasma proteins. We can differentiate between genetic defects and polymorphisms. In the first case the proteins may have an altered structure, so that they are biologically inactive. They may be still detectable by immunological techniques or they may be absent.

Table 18. Hereditary deficiencies of human plasma proteins.

Albumin	Transcobalamin II	Fibrinogen
α_1 -Antitrypsin	β -Lipoprotein	AHG (F VIII)
α_1 -Lipoprotein	C 3 component	Prothrombin
TBG	C 1 r component	Factor V, VII, IX, X
Haptoglobin	C 6 component	Hageman-factor
Cholinesterase	β_2 -Glycoprotein I	Prekallikrein
C 1-Inactivator	IgG	α_2 -Plasmin inhibitor
Ceruloplasmin	IgA	
C 2 component	IgM	
Transferrin		

Table 19. Plasma protein deficiency diseases.

α_1 -Antitrypsin	lung emphysema, neonatal hepatitis
α_1 -Lipoprotein	Tangier disease, enlarged yellow tonsils, hepatosplenomegaly, lymphadenopathy
C 1 Inactivator	angioneurotic edema
Ceruloplasmin	Wilson's disease, acute hemolytic anemia
Transferrin	severe hypochromic anemia
Transcobalamin II	severe neonatal megaloblastic anemia
—V B ₁₂ binding—	growth failure, repeated infections
β -Lipoprotein	steatorrhea, neuromuscular disorder
C 3, C 1 r, C 6	Impaired bactericidal activity
IgG, IgA, IgM	antibody deficiency diseases
Fibrinogen	Abnormal bleeding tendency
AHG (F VIII)	
Prothrombin	
Factor V, VII, IX, X	
Hageman-factor	
Prekallikrein	
α_2 -Plasmin inhibitor	

In the case of the polymorphism the alteration of the primary structure causes separate phenotypes, each of which has one appreciable incidence and where the biological activity is not impaired (Table 18).

Hereditary deficiency could be observed with more than 25 different plasma proteins, which are not necessarily connected with serious illness. The next table shows deficiencies connected with diseases (Table 19).

In the next table we have listed up human plasma proteins with known polymorphisms. The determination of the polymorphisms is made by electrophoretic and immunological techniques or by estimation of the function of the proteins (Table 20).

The reasons for some polymorphisms are known. They are due to amino-acid substitution or substitution or substitution of whole polypeptide-chains. In addition, differences in the carbohydrate moieties can be the basis for polymorphisms.

If we add to the plasma protein polymorphisms the numerous known enzyme polymorphisms in man, one can imagine that each person is unique in his protein and enzyme constitution, that means, he is characterized by his pattern of polymorphism as by his finger-prints.⁹⁷⁾

Table 20. Human plasma protein polymorphisms.

Albumin	Transferrin
α_1 -Antitrypsin	C 3 component
α_1 -Acid glycoprotein	C 4 component
Gc-component	C 3 proactivator
Cholinesterase	β -Lipoprotein
Haptoglobin	Fibrinogen
Ceruloplasmin	IgG, IgA, IgM

Final remarks

Ladies and gentlemen, this review about recent knowledge of human plasma proteins can be, of course, not complete. So for instance, I have excluded the foetal proteins, about which my good friend Prof. Hirai and many other Japanese colleagues are working intensively and successfully. I also did not go into the placental proteins and the proteins being associated with pregnancy, but I think I could show you that our knowledge in human plasma proteins has increased in the last years tremendously.

In the time, when Emil von Behring and Kitasato worked together very fruitfully, one publication of the Institute of Behring in Marburg showed that there are only four human plasma proteins (Fig. 19). Today we know of more than one hundred well-characterized human plasma proteins and I personally believe, we are still in the beginning of this research, especially with regard to the importance of the diagnostic of human plasma proteins within the clinic.

25 years ago, two famous Japanese professors presented this vase to our institute on the occasion of the 100th birthday of Emil von Behring. Let us work together, to understand more and more about human plasma proteins, in a friendly, harmonic way, as it is expressed by this beautiful Japanese vase and the German summer flowers (Fig. 20).

Fig. 19.

Fig. 20.

References

- 1) Schultze, H.E. and Heremans, J.F. : Molecular Biology of Human Proteins, Elsevier, Amsterdam, 1966.
- 2) Putnam, F.W. : in "Trace Components of Plasm" Alan R. Liss, Inc., New York, 1976.
- 3) Schultze, H.E. and Schwick, H.G. : Behringwerk-Mitteilungen No. 35, 57-73, 1958.
- 4) Koch, Fr., Schultze, H.E. and Schwick, H.G. : Klin. Wo., **36** : 17-20, 1958.
- 5) Haferkamp, O., Schlettwein-Gsell, D., Schwick, H.G. and Störiko, K. : Gerontologia, **12** : 30-38, 1966.
- 6) Haferkamp, O., Schlettwein-Gsell, D., Schwick, H.G. and Störiko, K. : Klin. Wo., **44** : 725-726, 1966.
- 7) Schwick, H.G. : in "Alter und Blutgerinnung," F.K. Schattauer, Stuttgart-New York p. 85-91, 1970.
- 8) Wecke, B. and Krasilnikoff, P.A. : Acta Med. Scand., **192** : 149-155, 1972.
- 9) Davie, E.W. and Hanahan, D.J. : "Blood Coagulation Proteins" in "The Plasma Proteins" Vol. III, (F.W. Putnam), Acad. Press New York, p.422-544, 1977.

(146) 生物物理化学

- 10) Sutti, J. W. and Jackson, C. M. : *Physiol. Res.*, **57** : 1-70, 1977.
- 11) Ratnoff, O. D. : *Behring Inst. Mitt.*, **63** : 135-155, 1979.
- 12) Vaheri, A. and Mosher, D. F. : *Biochim. Biophys. Acta*, **516** : 1-25, 1978.
- 13) Sodetz, J. M., Paulson, J. C., Pizzo, S. V. and Mckee, P. A. : *J. Biol. Chem.*, **253** : 7202-7206, 1978.
- 14) Mannucci, P. M. : *Diahn. and Intensivtherapie*, **6** : 49-51, 1979.
- 15) Müller-Eberhard, H. J. : *Behring Inst. Mitt.*, **61** : 1-13, 1977.
- 16) Porter, R. R. and Reid, K. B. M. : *Nature*, **275** : 699-704, 1978.
- 17) Lachmann, P. J. : *Behring Inst. Mitt.*, **63** : 25-37, 1979.
- 18) Laurell, A. -B. : *Laboratoriumsblätter*, **3** : 89-102, 1977.
- 19) Heimbürger, N. : in "Proteases and Biological Control" (E. Reich, D. Rifkin, E. Shaw, eds.) Cold Spring Harbor, New York, 1975.
- 20) Barrett, A. J., Brown, M. A. and Sayers, Ch. A. : *Biochem. J.* in press, 1979.
- 21) Hall, Ph. K. and Roberts, R. C. : *Bioch. J.*, **173** : 27-28, 1978.
- 22) Adham, N. F., Song, M. K. and Rinderknecht, H. : *Biochim. Biophys. Acta*, **495** : 212-219, 1977.
- 23) Travis, J., Bowen, J. and Baugh, R. : *Biochem.*, **17** : 5651-5656, 1978.
- 24) Orgasaeter, H. : *Act. Path. Microbiol. Scand. Sect. C*, **84** : 235-244, 1976.
- 25) Chawla, R. K., Wabsworth, A. D. and Rudman, D. : *J. Immunol.*, **121** : 1636-1639, 1978.
- 26) Aoki, N., Saito, H., Kamiya, T., Koie, K., Sakata, Y. and Kobakura, M. : *J. Clin. Invest.*, **63** : 877-884, 1979.
- 27) Travis, J., Garner, D. and Bowen, J. : *Biochem.*, **17** : 5647-5651, 1978.
- 28) Putnam, F. W. : in "The Plasma Proteins" Vol. III, (F. W. Putnam), Acad. Press New York, p. 2-284, 1977.
- 29) Jacobsen, Ch. : *Biochem. J.*, **171** : 453-459, 1978.
- 30) Schultze, H. E., Schönenberger, M. and Schwick, H. G. : *Bioch. Z.*, **328** : 267-271, 1956.
- 31) Peterson, P. A. : *J. Biol. Chem.*, **246** : 34-43, 1971.
- 32) Blake, C. C. F., Heide, K., Perat, C. and Swan, I. D. A. : *Acad. Sci.*, **272** : 195-198, 1971.
- 33) Blake, C. C. F., Geisow, M. J., Swan, I. D. A., Rerat, C. and Rerat, B. : *J. Mol. Biol.*, **88** : 1-12, 1974.
- 34) Cheng, Sh. -Y., Cahnmann, H. J. and Robbins, J. : *Biochem.*, **14** : 4132-4136, 1975.
- 35) Robbins, J. : in "Trace Components of Plasma" Alan R. Liss, Inc. New York, p. 313-350, 1977.
- 36) Daiger, St. P., Schanfield, M. S. and Cavalli-Sforza, L. L. : *Proc. Nat. Acad. Sci.*, **72** : 2076-2080, 1975.
- 37) Svasti, J. and Bowman, B. H. : *J. Biol. Chem.*, **253** : 4188-4194, 1978.
- 38) Stenmann, U. -H. : *Scand. J. Haemat.*, **14** : 91-107, 1975.
- 39) Wickramasinghe, S. N., England, J. M., Saunders, J. E. and Down, M. C. : *Acta Haemat.*, **54** : 89-94, 1975.
- 40) Neale, F. C., Aber, G. M. and Northam, B. E. : *J. Clin. Path.*, **11** : 206, 1958.
- 41) Schultze, H. E., Heide, K. and Haupt, H. : *Naturwissenschaften*, **48** : 696, 1961.
- 42) Müller-Eberhard, V. and Liem, H. H. : *La Ricerca Clin. Lab.*, **5** : 275, 1975.
- 43) Black, J. A. and Dixon, G. H. : *Nature*, **218** : 736-741, 1968.
- 44) Pastewka, J. V., Ness, A. T. and Peacock, A. C. : *Biochim. Biophys. Acta*, **386** : 530-537, 1975.
- 45) Fuller, G. M., Rasco, M. A., McCombs, M. L., Barnett, D. R. and Bowman, B. H. : *Biochem.* **12** : 253-258, 1973.
- 46) Poulik, M. D. and Weiss, M. L. : in "The Plasma Proteins," Vol. II, (F. W. Putnam), Acad. Press New York, p. 52-101, 1977.
- 47) Levy, R. I. : in "Trace Components of Plasma" Alan R. Liss, Inc., New York, p. 25-41, 1976.
- 48) Scanu, A. M., Edelstein, C. and Keim, Ph. : in "The Plasma Proteins" Vol. I, (F. W. Putnam), Acad.

Press New York, p.317, 1975.

- 49) Faergeman, O. : Acta Med. Scand., **203** : 614, 1978.
- 50) Barragry, J.M., Corless, D., Auton, J., Carter, N.D., Long, R.G., Maxwell, J.D. and Switala, S. : Clin. Chim. Acta, **87** : 359-365, 1978.
- 51) Hesh, R.-D., Gatz, J., McIntosh, C.H.S., Janzen, J. and Hehrmann, R. : Clin. Chim. Acta, **70** : 33-42, 1976.
- 52) Ingenbleek, Y., De Nayer, Ph. and De Vissher, M. : JCE and M, **39** : 179-180, 1974.
- 53) Schmid, K., Nimberg, R. B., Kimura, A., Yamaguchi, H. and Binette, J. P. : Biochim. Biophys. Acta, **492** : 291-302, 1977.
- 54) Schmid, K., Emura, J., Schmid, M.F., Stevens, R.L. and Nimberg, R.B. : Int. J. Peptide Protein Res., **11** : 42-48, 1978.
- 55) Gahmberg, C.G. and Andersson, L.C. : J. Exp. Med., **148** : 507-521, 1978.
- 56) Piafsky, K.M. and Borgo, O. : Clin. Pharmacol. and Therap., **22** : 545-549, 1978.
- 57) Snyder, St. and Coodley, E.L. : Arch. Intern. Med., **136** : 778-781, 1976:
- 58) Haupt, H. and Heide, K. : Clin. Chim. Acta, **10** : 555-558, 1964.
- 59) Schultze, H.E., Heide, K. and Haupt, H. : Nature (London), **200** : 1103, 1963.
- 60) Haupt, H. and Heimbürger, N. : Hoppe Seyler's Zeitschr. Physiol. Chem., **353** : 1125-1132, 1972.
- 61) Binette, P. and Binette, M. : Biochem. J., **143** : 253-254, 1974.
- 62) Pinteric, L., Assimeh, S.N., Kells, D.I.C. and Painter, R.H. : J. Immunol., **117** : 79-83, 1976.
- 63) Osmand, A.P., Friedenson, B., Gewurz, H., Painter, R.H., Hofmann, Th. and Shelton, E. : Proc. Natl. Acad. Sc. U.S.A., **74** : 739-743, 1977.
- 64) Bürgi, W. and Schmid, K. : J. Biol. Chem., **236** : 1066-1074, 1961.
- 65) Schultze, H.E., Heide, K. and Haupt, H. : Naturwissenschaften, **49** : 15, 1962.
- 66) Triffitt, J.T., Gebauer, V., Ashton, B.A., Owen, M.E. and Reynolds, J.J : Nature, **262** : 226-227, 1976.
- 67) Heimbürger, N. Haupt, H., Kranz, T. and Baudner, S. : Hoppe-Seyler's Z. Physiol. Chem., **353** : 1133-1140, 1972.
- 68) Morgan, W.T., Koskelo, P., Koenig, H. and Conway, Th. P. : Proc. Soc. Exp. Biol. Med., **158** : 647-651, 1978.
- 69) Morgan, W.T. : Biochim. Biophys. Acta, **533** : 319-333, 1978.
- 70) Haupt, H., Baudner, S., Kranz, T. and Heimbürger, N. : Europ. J. Biochem., **23** : 242-247, 1971.
- 71) Iwasaki, T. and Schmid, K. : J. Biol. Chem., **245** : 1814-1820, 1970.
- 72) Haupt, H. and Baudner, S. : Hoppe-Seyler's Z. Physiol. Chem., **358** : 639-646, 1977.
- 73) Svensson, L. and Ravnskov, V. : Clin. Chim. Acta, **73** : 415-422, 1976.
- 74) Ekström, B. and Berggord, I. : J. Biol. Chem., **252** : 8048-8057, 1977.
- 75) Takagi, K., Kin, K., Itoh, Y., Kawai, T., Kasahara, T., Shimoda, T. and Shikata, T. : J. Clin. Invest., **63** : 318-325, 1979.
- 76) Seon, B.K. and Pressman, D. : Biochemistry, **17** : 2815-2821, 1978.
- 77) Berggord, I. and Bearn, A.G. : J. Biol. Chem., **243** : 4095-4103, 1968.
- 78) Poulik, M.D. : in "Trace Components of Plasma" Alan R. Liss, Inc. New York, p.155-177, 1976.
- 79) Hammerschmidt, D.E. and Moldow, Ch.F. : J. Lab. Clin. Med., **92** : 1002-1008, 1978.
- 80) Haupt, H. and Heide, K. : Clin. Chim. Acta, **14** : 418-421, 1966.
- 81) Haupt, H., Schwick, H.G. and Störiko, K. : Humangenetik, **5** : 291-292, 1968.
- 82) Burstein, M. and Legman, P. : Protides of Biol. Fluids, **25** : 407, 1977.
- 83) Schwick, H.G., Haupt, H. and Heide, K. : Klin. Wschr., **46** : 981-986, 1968.

(148) 生物物理化学

- 84) Chase, W.H. and Prochaska, H. : Clin. Immunol. Immunopath., **5** : 247-257, 1976.
- 85) Oliveira, E.B. Gotschlich, E.C. and Lin, T.-Y. : J. Biol. Chem., **254** : 489-502, 1979.
- 86) Osmand, A.P., Friedenson, B., Gewurz, H., Painter, R.H., Hofmann, T. and Shelton, E. : Proc. Natl. Acad. Sci. U.S.A., **74** : 739-743, 1977.
- 87) Marchalonis, J.J. and Weltman, J.K. : Comp. Biochem. Physiol. B. Comp. Biochem., **38** : 609-625, 1971.
- 88) Gale, K. and Miltenyi, M. : Acta Microbiol. Acad. Sc. Hung., **3** : 41-51, 1955.
- 89) Ganrot, P.O. and Kindmark, C.-O. : J. Lab. Clin. Invest., **24** : 215-219, 1969.
- 90) Heide, K. and Seiler, F. : Arzneimittel-Forschg., **21** : 1443-1446, 1971.
- 91) Fiedel, B.A., Simpson, R.M. and Gewurz, H. : J. Immunol., **119** : 877-882, 1977.
- 92) Heide, K. and Schwick, H.G. : Angew. Chem., **85** : 803-815, 1973.
- 93) Springer, G.F., Schwick, H.G. and Fletcher, M.A. : Proc. Nat. Acad. Sci. U.S.A., **64** : 634, 1969.
- 94) Simpson, D.L., Thorne, D.R. and Loh, H.H. : Life Sciences, **22** : 727-748, 1978.
- 95) Uhlenbruck, G., Baldo, B.A., Steinhausen, G., Schwick, H.G., Chatterjee, B.P., Horejsi, V., Krajhansl, A. and Kocourek, J. : J. Clin. Chem. Clin. Biochem., **16** : 19-23, 1978.
- 96) Uhlenbruck, G., Karduck, D., Haupt, H. and Schwick, H.G. : Z. Immun. Forschg., **155** : 262-266, 1979.
- 97) Harris, H. : Canad. J. Genet. Cytol., **13** : 381, 1971.
- 98) White, A. and Durton, P. : Conf. on Subcell. Factors in Immunity, 26.2.-1.3.1979, New York, 1979.