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DEPARTMENT OF THE ARMY  
Fort Detrick  
Frederick, Maryland

## INFECTIOUS DISEASES AND PLASMA PROTEINS

Naika (Tokyo) 15;1076-86.  
June, '66

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The changes in fraction or subfraction of plasma proteins have been observed in various infectious diseases. However, the meaning of these changes has not been well understood yet. In this article, an attempt was made to materialize these informations from both ~~my~~ experience and publications in the past. Furthermore, a recent progress in biochemical findings of the antibody, especially of its specificity was described. In last, some discussion was extended to the "Antibody Deficiency Syndrome" of which gamma globulin concentration in plasma was abnormally low.

### I. INTRODUCTION

Some investigators in the past had tried to correlate the changes of the plasma protein concentration with the changes of its electrophoretic fractions in the certain diseases and classify its results into a certain pattern for the aid of diagnosis. 1-3

Since recent progress has been made in physiochemical, immunological fields (i.e.; starch gel electrophoresis, agar-gel electrophoresis, ion exchange chromatography, gel filtration, immune electrophoresis and so on), the endless details of changes of fractions, subfractions of plasma proteins have been continuously reported.

Among the several clinical, pathological entities, the relationship between the infection and changes of plasma pro-

tein fractions, subfractions are to be discussed together with the relationship between infection and antibody deficiency syndrome in the next paragraph.

## II. CHANGES OF THE PLASMA PROTEIN FRACTION IN INFECTION

Wuhrmann and Wunderly<sup>1</sup> correlated the changes of the plasma proteins with the findings of the red sedimentation rates, the corrosive sublimate reaction, cephalin-cholesterol flocculation test, thymol turbidity test, cadmium reaction, total amount of protein and heat-coagulation test.

From the result of their study, they formed the "REACTIONS CONSTELLATION" theory in which, they believed, some diseases could be classified into certain patterns according to the changes of plasma proteins. In this theory, the pattern was classified in nine types, starting from an acute inflammatory type and ending to beta globulin plasmocytoma. The infectious diseases were categorized in Type I, Acute inflammatory type, Type II, Subacute-chronic inflammatory and proliferative type (Type III, Hepatitis type as a specific type). The changes of plasma proteins of Type I, II, III are illustrated in Table I. As seen in Table I, no essential difference is found between Type I and II; i.e.; a decrease in albumin, increase in alpha-2, beta, gamma globulin were seen in both types. But a degree of these changes are more prominent in Type I than in Type II. Also, in Type I, the changes of beta globulin is more dominant in Type II than Type I. In Type III, no significant change of alpha globulin is observed. Miyoshi<sup>2,3</sup> criticized that the classification made by Wuhrmann and Wunderly was not only phenomenological but also did not always satisfy the entity of the disease. Instead, he advocated the concept of "Protein disease" or "Blood protein disease". According to him, the diseases of which pathological etiology or cardinal symptoms are due to abnormality of either protein or of plasma protein can be categorized to nine groups. He stated that an alternation of plasma protein observed in infectious diseases possessed a peculiar pattern which was deemed as the "Infectious disease pattern". In Fig. 1, a transitory change of each plasma protein fraction of the infectious disease pattern is illustrated. As seen in Fig. 1, together with a slight decrease of albumin, an increase of each fraction (including fibrinogen) in an acute stage is noticed but as it becomes chronic, both a decrease of albumin and an increase of gamma globulin becomes more prominent. The changes of alpha- and beta globulins are not marked. When the chronic stage is prolonged, so called "status hypergammaglobulinemicus" is observed as illustrated in the right bottom of Fig. 1.

TABLE I. SUMMARY OF PLASMA PROTEIN CHANGES (REACTION CONSTELLATION)  
(from Wuhrmann and Wunderly<sup>1</sup>)

	Acute Inflammatory type	Chronic Inflammatory type	Hepatitis type
Red cell stimulation	marked	marked or slight (can be normal)	no change or intermediate
Corrosive Sublimate Reaction	negative	negative	positive (60-70%)
Thymol Turbidity	negative	negative	positive
Cadmium Reaction	positive	positive (-) when B-1-g elevated	positive
Total Protein	No change or slight increase decrease A/G ratio.	normal, decreased albumin, increased gamma globulin	decrease, decreased albumin increased gamma globulin
Heat coagulation	Elevated, narrowed Weltman band. Marked left shift of nephelogram with widened bottom.	Slightly narrowed Weltman band. Lowered nephelogram with normal bottom.	Widened Weltman band. Right shifted, narrowed lowered nephelogram.
Electrophoresis	marked increase in alpha-2 globulin, decreased albumin. Later gamma globulin increased.	intermediate or marked decrease in albumin, increased gamma globulin sometimes increased alpha-2, beta globulin	increased gamma globulin. Decreased albumin, non change of alpha g. slight elevation of beta g.
Example	-Pneumonitis -Initial stage of infection -Exudative type of pulmonary TB -Acute polyarthritis, Sepsis, phlegmon.	-Recovery from pneumonitis -Recovery from acute infection -Chronic PFB -Other chronic infectious diseases	hepatitis

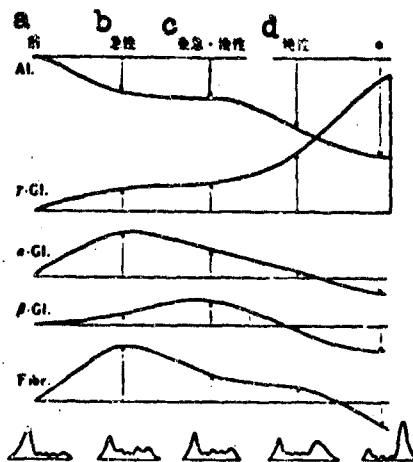


Fig. 1. Transient Changes of Plasma Protein Fractions in Infectious Diseases (from Miyoshi<sup>3</sup>)  
 Legend: a. before disease; b. acute stage;  
 c. subacute, chronic stage; d. chronic stage

A general survey on changes of plasma proteins is as mentioned above. Some detailed changes of each fraction, sub-fraction of plasma protein in an infectious disease is to be described except alpha and gamma globulins in the next paragraph. Alpha-globulin is to be discussed as an acute phase reactants and gamma-globulin is to be discussed as the changes of immunoglobulin later.

#### A. Albumin

The common findings among the several published papers dealing with the relationship between the infectious diseases and plasma proteins are an absolute or relative reduction of serum albumin.

Jenkins<sup>4</sup> reported that a prominent decrease of albumin in 22 cases of pulmonary tuberculosis, 11 cases of pyelonephritis and a slight decrease of albumin in the 11 cases of pneumonitis, 12 cases broncho-pneumonitis. Graham et al<sup>5</sup> stated that an absolute or relative reductions of albumin were noticed in the 40 cases of the various infectious diseases and 17 cases of virus infectious. Furthermore, Brackenridge<sup>6</sup> reported the similar findings of total 86 cases, including 53 cases of acute infectious diseases such as the 43 cases of pneumonitis, gonorrhea, abscess, panperitonitis; 10 cases of pulmonary tuberculosis 3 cases of chronic otitis media, 4 cases of bacterial endocarditis and 25 cases of

TABLE II. CHANGES OF PROTEIN, FRACTION  
CONCENTRATION IN PLASMA AT INFECTION  
(from Jenoks<sup>4</sup>)

	Number of Cases	albumin, (%)	alpha- 1- g (%)	alpha- 2- g (%)	beta- g (%)	gamma- g (%)	Total pro- tein g/dl
Normal human	185	68.9	2.9	7.3	9.0	12.0	7.3
Pulmonary tuberculosis	22	59.6	4.0	9.5	9.9	17.0	7.0
Pneumonitis	11	65.0	4.1	8.8	9.7	12.4	6.9
Bronchopneu- monia	12	64.3	3.9	10.0	9.6	11.9	6.7

chronic bacterial infections such as osteomyelitis, tuberculosis, lymphadenitis, or viral infection, infectious mononucleosis, malaria.

Generally, hypoalbuminemia is considered due to either a decreased albumin synthesis (i.e.; liver disease) or an increased catabolism or a pathologically increased excretion of albumin, besides a genetic factor, malnutrition or an increased excretion of albumin. In the bacterial infection, it seems that the bacterial toxin promotes the catabolism of protein by tissue destruction, resulting in hypoalbuminemia eventually. Also, in mice experimentally induced by the lipopolysaccharide of *Serratia marcescens*, a hypoalbuminemia was noticed.<sup>7</sup> On the contrary, in the experiment of which the protein synthesis in the liver, spleen and lymph node of the staphylococcus infected mice was studied by an autoradiography of the immune electrophoresis, the albumin synthesis was found increased.<sup>8</sup> This was contradictory to the reduction of albumin in the circulation mentioned before. The contradictory findings can be explained as due to the increased catabolism of the synthesized albumin and its extravascular leakage in an acute inflammatory stage. However, it requires furthermore investigation.

It is well known that at the early stage of the infection, the serum albumin is increased temporarily as a stress reaction is monitored through the hypophyseoadrenocortical system. However, it is equivocal that William's finding is due to stress reaction.

## B. Transferrin

Transferrin (Siderophilin) is an iron-combined protein belonging to beta-I-globulin and its molecular weight is 90,000. 1 mg. of the transferrin combines with 1.32  $\gamma$  of iron. It is well known that the iron combining power is reduced in spite of the low serum iron concentration at the infection, but its mechanism has not been well clarified yet. The iron combining power is a clinical expression of the body transferrin content and in case of genuine iron deficiency, it is found increased remarkably.

The authors<sup>9</sup> investigated the metabolism of transferrin by use of  $^{131}\text{I}$  labelled transferrin; In infection, a reduction of blood transferrin concentration, a marked shortening of its half life, and a markedly increased turnover rate were recognized. On the other hand, a synthesis of transferrin was found decreased in the liver of the infected animal. Therefore, the cause of the iron combining power depletion at the infection was due to both the increased catabolism and the decreased synthesis of transferrin. Furthermore, we demonstrated that the transferrin concentration in the abscessed testicle of rat caused by terpenin artificially was increased to eight to sixteen times of the normal one. Thus, it was proved that the localization of transferrin to the infected foci was one of the cause of the blood transferrin reduction.<sup>9b</sup> The above mentioned data may be related to the antibacterial action of the free transferrin found in vitro<sup>10</sup> and clinically.<sup>11</sup> Extensive study about this problem is being carried on.

## III. ACUTE PHASE REACTANTS

Many of the plasma responses to acute inflammation including an acute infection are non-specific; i.e., the increased blood sedimentation rate. These reactions are summarized as the "Acute phase phenomenon"<sup>12</sup> and the reactants are called as the "Acute phase reactants" which include C-reactive protein, fibrinogen and the other several glycoproteins.

### A. Alpha-globulin

Alpha-globulin was divided into only two fractions; i.e. alpha-1 and alpha-2-globulin when Teselius or paper electrophoresis was utilized for analysis. But, as progress was being made in the field of the immune<sup>13,14</sup> or the starch gel electrophoresis,<sup>15-17</sup> several subfractions of alpha-globulin had been discovered. The tabling of the known subfractions of alpha-globulin were prepared by Taniuchi<sup>18-19</sup> or Uda.<sup>20</sup> Nevertheless, among the total fifteen or seventeen subfractions



of alpha-1 or alpha-2 globulins, the functions of not a few of these are not clarified yet. Wada<sup>21</sup> summarized that the main changes of the subfractions in an inflammatory disease were the increase of orosomucoid, alpha-1-glycoprotein, alpha-1-HL2 (thermolabile factor) in the alpha-1-globulin region, and the increase of haptoglobin, ceruloplasmin in the alpha-2-globulin region. He stated, furthermore, that the total increase of alpha-1 alpha-2 globulins were mainly influenced by the increase of orosomucoid, alpha-1-glycoprotein in the alpha-1 region and by the increase of haptoglobin in the alpha-2-region. Since the significance of orosomucoid is almost identical to one of alpha-1-glycoprotein,<sup>24</sup> these subfractions are discussed together in the next paragraph.

#### 1. Orosomucoid<sup>22</sup> (Alpha-1-seromucoid or alpha-1-acid glycoprotein<sup>23</sup>) and alpha-1-glycoprotein.

The precipitation constant of orosomucoid is said 3.118<sup>22</sup> or 3.55<sup>26</sup> and its molecular weight is 44,000<sup>25</sup> while the precipitation constant of alpha-1-glycoprotein is 3.55 and its molecular weight is 54,000. The increase of these substances at an infection were reported somewhere<sup>28</sup> and Betsueki recently made an observation of the transitory change of these subfractions in detail<sup>24</sup>. Both substances must be regarded as the important acute phase reactant because the responses of these substances to acute inflammations are sharp. However, neither the origins of these substances nor their physiological pathological significances are clarified yet at present time. An increase of glycoprotein such as orosomucoid or alpha-1-glycoprotein at an inflammation may be interpreted as a stress reaction motivated through the hypophysis-adrenocortical system because of its non-specific nature. The fact that a depletion of glycoprotein in either hypophysial or adrenocortical insufficiency may support the above findings.<sup>29</sup> However some reported the glycoprotein concentration of the normal guinea pig<sup>30</sup> and the dog were not altered by the intramuscular injection of ACTH or cortisone. Therefore no conclusion was drawn yet about this problem.

#### 2. Haptoglobin

Haptoglobin is one of the glycoprotein which belongs to alpha-2-globulin and forms a stabilized combination with hemoglobin. Its genetic types as well as the one of transferrin were studied thoroughly<sup>15,16</sup>. But its genetic problem must be spared for the sake of the economy of space.

The precipitation constant of its type 1~I is 4.25<sup>32</sup> and its molecular weight is 85,000<sup>33</sup>. A few different

techniques had been used to measure haptoglobin, i.e., Connell and Smithies' method<sup>34</sup> is based on the measurement of the peroxidase activity of Hp-Hb complex, while Laurell and Nyman's method<sup>33,35</sup> is based on electrophoresis. Its normal blood concentration limit is  $\pm 100$  mg/dl.<sup>33,36</sup> (30~186 mg/dl according to Betsueki<sup>37</sup>) Wada reported<sup>38</sup> that eight out of the fifteen acute inflammatory cases (including five out of the six rheumatic fever cases) studied by him revealed significant rises of their haptoglobin level. However, a metabolic change of haptoglobin during infection is not well studied yet.

### 3. Slow Alpha-2

When the serum of healthy human being was analyzed by starch gel electrophoresis at pH 8-8.5, a single distinct line which is called slow alpha-2 is obtained. It is well known that slow alpha-2 as well as transferrin is useful for a fixation of the electrophoretic pattern. It has not been known yet which subfraction demonstrated by the immune electrophoresis is correspondent to the line representing the slow alpha-2. However, it has been thought that slow alpha-2 is represented by the mixture of alpha-2 macroglobulin, fibrinogen and so on.<sup>18</sup> The slow alpha-2 is not observed in the normal rat, but appears in the experimentally infected rat.<sup>39,40</sup>

When the endotoxin such as lipopolysaccharide deprived from the horse abortus bacilli was injected into the abdominal cavity of rat, the slow alpha-2 was recognized at 2nd-9th day. Alpha-2 macroglobulin as well as orosomucoid is regarded as one of the acute phase reactants. However, the origin of glycoprotein seen in an acute infection or inflammation had been said either due to the destruction of tissue or due to the proliferation of tissue, which had been undecided yet. No observation regarding its origin was made in Heim's experiment,<sup>39,40</sup> nevertheless, it is clear that it is one of the acute phase reactants.

### B. C-reactive protein

Tillett et al<sup>41</sup> reported the observation that the substance in the serum of the pneumococcal pneumonitis patient was reacted with C-polysaccharide of the pneumococci bacilli, causing the sedimentation. This substance is called C-reactive protein (CRP). CRP is found not only in the serum of pneumococcal pneumonitis but also in the one of acute bacterial infection<sup>42-45</sup>, parasitic diseases and in one of the viral diseases.<sup>47</sup> Especially, CRP appears in a high percentage in the serum of an acute rheumatic fever.<sup>48,49</sup> Therefore, its diagnostic value is not only significant but also it is considered valuable as the judgement of its prognosis be-

cause the transitory change of CRP level is well paralleled with the activity of rheumatic fever. CRP has been extensively used for clinical purpose nowadays.

The chemical, physical nature of CRP<sup>50,51</sup> is entirely different from the antibody because it does not exist in a serum of the healthy subject,<sup>50</sup> and it requires calcium as the reacting medium.<sup>42</sup>

CRP is precipitated from the serum after 50%-70% saturated sodium sulfate or ammonium sulfate is added. The precipitation of CRP is also observed when it is filtered through the running water or 0.02% Calcium chloride, but it is soluble in distilled water. It was reported that CRP existed in the blood as combined with lipid.<sup>51</sup> Though the report is past<sup>5</sup> indicated that the mobility of CRP in the electrophoresis was similar either to the one of the beta globulin or to gamma globulin, the recent study indicated that it is situated between one of the alpha-2 and of the beta globulin.<sup>54</sup> Its precipitation constant is percent 7.5 S and its electric point is 4.8.

CRP is detected by the use of its specific reaction to the C-polysaccharide or by the use of its action to induce the swelling of the capsular membrane of the pneumococcus bacilli. But for a quantity measurement, the immunochemical technique, that is, the technique to utilize the anti-CRP rabbit serum is regarded as the more sensitive and accurate method. This technique is reported in detail by Homma.<sup>57</sup> It was already mentioned that CRP was one of the acute phase reactants. Rice<sup>58</sup> investigated the relationship of CRP to ceruloplasmin, sialic acid, seromucoid and total glycoprotein hexose by measuring of all the substances quantitatively at the same time. According to his investigation, the positivity of CRP is closely related to the increase of other four; In other words, all the other substances can be regarded as the acute phase reactants, and especially CRP is most closely related to sialic acid among the four substances.

#### IV. INFECTION AND IMMUNOGLOBULIN

The evidence that the relative and absolute increase of alpha globulin seen in several infectious diseases reflects the production of the antibody. Groso<sup>59</sup> summarized names of the infectious diseases in which the gamma globulin were reported increased.

Whurmann and Wunderly<sup>1</sup> reported the transitory change of gamma globulin together with the change of other sub-fractions in each infectious diseases in detail.

Petermann<sup>60</sup> also reported the general survey about this subject. Therefore, in this paragraph, no further description is avoided except illustrates Gross's table (see Table 3). Instead, the specificity of antibody produced in the body is to be discussed in the next paragraph since several papers about this subject has been presented recently.

TABLE 3. INFECTIOUS DISEASES ACOMPANYING  
HYPERGAMMAGLOBULINEMIA  
(from Gross<sup>59</sup>)

- 
1. Bacterial
    - a. Streptococcal endocarditis, rheumatic fever, acute glomerulonephritis
    - b. Severe staphylococcal infection
    - c. Advanced tuberculosis (pulmonary and other)
    - d. Tubercloid leprosy
  2. Spirochetal-
    - a. Syphilis
  3. Viral
    - a. Lymphogranuloma inguinale
    - b. Infectious mononucleosis
    - c. Psittacosis
  4. Rickettsiae
    - a. Typhus fever
  5. Deep mycosis
    - a. Histoplasmosis
  6. Protozoa
    - a. Kala azar
    - b. Mucocutaneous Leishmaniasis
    - c. Malaria
  7. Nematoid
    - a. Visceral larva migrans
    - b. Trichinosis
  8. Non-specific chronic infectious disease

As a progress was being made in the field of plasma protein fixation by the immune electrophoresis, it became apparent that many subfractions had been deprived from gamma globulin which had been defined as a single entity by Tiselius or the paper electrophoresis. Among these, the functions of some subfractions are unknown. But, beta-2A, beta 2-M, and gamma-2-globulin possess the actions of antibodies. Consequently, these are called gamma-1A, 1M-globulin each. Hermans<sup>61</sup> suggested that these globulins should have been called as Immunoglobulin because of the physiological, chemical and immunological similarities. The nomenclature "Immunoglobulin" became quite popular at present and its various nature is illustrated in Table 4.<sup>62</sup>

Most immune antibodies produced in infectious diseases are originated from alpha-2-globulin, but some belongs to alpha-1M or alpha-1A globulins. The manners of the distribution of these antibodies in the immunoglobulin was well described in the references 20, 62-65. According to these references, the causative organisms producing the antibody which belongs to the alpha-2-globulin only are Escherichia coli, Shigellae and Typhoid H. The other antibody producing organisms are found distributed in two or three different kinds of immunoglobulins.

In the immune electrophoresis, regardless of the origin of the specimen, the curve representing the gamma-2-globulin is traced as a long arc extending from the position of the gamma globulin to the one of the beta or alpha globulin. Also, it is well known that in the starch gel electrophoresis, the gamma-2-globulin curve is traced markedly diffused compared to the other tracing band. This phenomenon is interpreted as the heterogeneity.

The heterogeneity of the gamma-2-globulin is said due to the conglomeration of the protein molecules which mobilities are slightly different from each other. However, these proteins cannot be identified by other criteria such as an ultracentrifuging or an antigen analysis. The difference of its mobility is due to the difference of electric charge of the individual protein molecule in the electric field of the immune electrophoresis; in other words, due to the configured difference of the alpha-2-globulin structure.

On the other hand, the abnormal protein observed in myeloma or Waldenstrom's macroglobulinemia and Bence Jones protein which is considered being extracted as L-chain of these abnormal protein<sup>66</sup> are indeed homogenous, being different from the pattern of the normal alpha-2-globulin.

TABLE 4. PHYSICO-CHEMICAL IMMUNOLOGICAL NATURE  
OF IMMUNOGLOBULIN  
(from Fohey<sup>62</sup>)

	Gamma 2-glob- ulin	Gamma 1A-glob- ulin	Gamma 1M- globulin
<u>Physiochemical</u>			
Molecular weight	160,000	(160,000)n	1,000,000
Precipitation constant (S <sub>20,w</sub> )	6.6S	6.6S 13S	19S
Electrophoretic mobility	gamma 2	gamma 1	gamma 1
Content of Carbon hydrate(percent)	2.6	10.7	12.2
Hexose	1.2	4.8	6.2
Hexosamine	1.1	3.8	3.3
Sialic acid	0.2	1.7	2.0
<u>Antigenicity</u>			
Specific antigen	+ (gamma 2)	+ (gamma 1A)	+ (gamma 1M)
Common antigen	+	+	+
<u>Immunological</u>			
Specific antibody	+	...	+
Placenta trans- mission	+	0	0
Skin fixation	+	0	0
Reaction with rheumatic factor	+	0	0
<u>Genetic</u>			
GM. factor	+	0	0
Inv. factor	+	+	+
g. percent in normal serum	1.2	0.4	0.1

The concept "clone" is indispensable when the manner of the productivity, the specificity of the antibody is to be discussed. The clone is represented by each individual group of antibody-producing cells. These cells develop into cell group which produces only single kind of antibody in the process of which the lymphatic cells differentiate and undergo

the various process of variation, becoming the antibody producing cell in the foetus. Waldenstrom<sup>67</sup> used the concept of clone to explain the homogeneity of the abnormal protein encountered in myeloma or Waldenstrom's macroglobulinemia. In these diseases, a single clone multiplies itself in proportionally, therefore the produced gamma-globulin is extremely homogenous. On the other hand, the normal gamma-2-globulin is the summation of the gamma-globulin produced by the whole clones.

Poulik and Edelman<sup>68</sup> reduced the proteins of the myeloma cases and the factor II-gamma-globulin of the healthy human being to the alkyl form. These specimens were studied under the starch gel electrophoresis containing 8M urea. Their study revealed that H or L chain obtained from myeloma protein and each myeloma protein demonstrated individual difference in their reaction. But the reduced form of the factor II gamma-globulin obtained from the healthy one did not. The result mentioned above can be explained well by the concept of clone. It seems that the immunological specificity of the various antibody (gamma-globulin) detected in the human sera is corresponding to the specific feature of the clone producing its antibody. In next, it must be questioned what makes the specificity of antibody (specificity of its combining site) being so numerously variable. By quoting Smithies' theory of the somatic rearrangement in the structural genes for gamma-globulin in the process of cell differentiation,<sup>70</sup> Edelman<sup>69</sup> explained it as follows. That is, the understanding of the proceeding such as a variation of the hereditary nature of the antibody producing cell → variety of the variation of amino acid sequence in producing H, L chains of gamma globulin (not only at the combining site, but also at the neighboring area.) → the variety of H, L chain combination and of its additional bonds would lead to explain the multifarious specificities of the antibodies.

## V. ANTIBODY DEFICIENCY SYNDROME

The group of infectious diseases in which severe infections are repeated because of lack of gamma-globulin is classified as "Antibody-deficient syndrome". In this paragraph, agammaglobulinemia and heavy chain disease are described as the typical examples of antibody-deficient syndrome. Myeloma, chronic lymphocytic leukemia and malignant lymphoma are also included in the group though the discussion about these diseases is omitted for the sake of economy of space.

### A. Agammaglobulinemia

It has been ten years or more already since the first time agammaglobulinemia was reported.<sup>71</sup> In these ten

years, a numerous finding concerning this disease has been progressively accumulated. At present, agammaglobulinemia is classified as primary and secondary and the primary one further divided into congenital and acquired. However, regardless of the classification, gammaglobulin concentration in plasma is reduced, consequently, severe infection is repeatedly affected in agammaglobulinemia.

### 1. Congenital agammaglobulinemia

This condition is a sex linked recessive defect, appearing only in males. It is usually detected in children from infancy; they are subject to repeated infections, usually bacterial (Staphylococcus aureus, Pneumococcus, Streptococcus, Meningococcus, Hemophilus influenzae and so on.) Infection due to gram negative bacilli is relatively rare. The clinical picture is variable. A large portion of these children often exhibits pneumonitis, meningitis and sepsis. However, otitis, sinusitis are more frequent. The gamma globulin level in blood of the normal healthy human being is approximately 0.6-1.5 g/dl, but in this condition, it is extremely low.; 0.05-0.15 g/dl or lower.<sup>75</sup> The reason of gammaglobulin reduction is due to a reduced or non-production of gamma-globulin despite antigen stimulation. However, the delayed type hypersensitivity is often maintained well. This phenomenon is demonstrated by 2-4 dinitrofluorobenzene,<sup>76</sup> tuberculin,<sup>77</sup> and histoplasmin.<sup>78</sup> The case reported by Aiya,<sup>71</sup> Pediatrics Department, Kyushu University Hospital showed a definite BCG change to the positivity.

### 2. Acquired agammaglobulinemia

This condition appears in both sexes. The primary symptom manifested as infection is seen from children to adulthood. The acquired type is either spontaneous or secondary one acquired after lymphatic granuloma or other tumor.

Concerning sex or age, it is different from the congenital type, but in both types, repetition of infection and the common type of the causative organism are observed. Pneumonitis is most common, while encephalitis, sprue-like syndrome and ulcerative colitis has been reported. In most cases, the onset of infection is at the age of seventeen to fifty four years old. One of the acquired type reported is agammaglobulinemia accompanied by the tumor of thymus gland.<sup>80</sup> This condition is interesting because thymus gland may play an important role in mechanism of immunization. However, it is too extensive a subject to be discussed in this short paragraph. Therefore, Miller's general review about this



subject<sup>81</sup> must be read by those who are interested.

### 3. Transient hypogammaglobulinemia

In the latter period of pregnancy, gammaglobulin of the mother is transmitted to the foetus through the placenta. It is interesting to know a percentage of transmission of gamma-globulin through placenta is about the same as albumin which molecular weight is far smaller than globulin, and is better than transferrin.<sup>82</sup> In a newborn baby, gammaglobulin is not produced and only the destructive process of gammaglobulin is being undertaken. Consequently, when the initiation of the production of gammaglobulin is delayed in newborn baby, gamma-globulin concentration in blood declines continuously. This is called transient hypogammaglobulinemia. Therefore, by affecting infection, a severe illness is inevitably followed in this condition. However, hypogammaglobulinemia in a newborn period is generally transient. As soon as a production of gammaglobulin is initiated, its blood concentration begins to increase.

It is known that the gammaglobulin-producing cells (plasma cells) are deficit in the lymph node, spleen and bone marrow of the agammaglobulinemic patients.<sup>83-86</sup> Therefore, it had been thought that no antibody was produced in this condition, no matter how much stimulation was given to the body. Recently, Fudenberg<sup>87</sup> presented a radically different view. According to him, agammaglobulinemia is not produced because of genetic defect in lymphatic or plasma cell system but because of genetic defect of gammaglobulin. He postulated also that the developmental differentiation from lymphatic cell to plasma cell is not always necessary for a production of the antibody, instead, it should have been considered as an accompanying phenomenon when antibody was produced, rather.

Should a morphological change of those cells after the antigen stimulation be considered to be the mere accompanying phenomenon, while gamma globulin (antibody) is in a process of production, the incomplete production of polypeptide chain of immunoglobulin would be the primary cause of agammaglobulinemia.

Such a possibility would be as follows:

a. The unexpected mutation of regulator gene loci which controls the production of polypeptide chain of immunoglobulin.

b. A loss of multiplication of genetic substance as a result of unequal crossing of chromosome which gene con-

trols the structure of gamma globulin at the developmental differentiation of cell.

c. The unexpected mutation of the gene which controls the structure of immunoglobulin itself.

In order to prove the above mentioned hypothesis, the antigens consisted of the diptheria toxoid, tetanus toxoid, typhoid antigen were injected to the five agammaglobulin cases and one hundred normal human beings, then their sampled leucocytes were culture in vitro. When stimulated by the antigen, the developmental differentiation from lymphatic cells to plasma cells, followed by the production of gammaglobulin were observed in the normal group, but not in the agammaglobulinemic group. Especially when Streptomycin O was added to the culture media, the production of antibody, enlargement of lymphatic cell or development of plasma cell were seen in all the normal group, but not at all in the agammaglobulinemia group. Furthermore, when Streptomycin S, phytochemagglutinin, as a non specific antigen stimulant, were added to the culture media, the enlargement of lymphatic cell and the development of plasma cell were observed in both groups while RNA and three different kinds of immunoglobulin were produced only in the normal group. In the agammaglobulinemia group, no production of gammaglobulin was observed.

From the findings mentioned above, Fudenberg made hypothesis that this condition was created not because the body was unable to respond to the specific stimuli due to the lack of plasma cells, but because the plasma cells were lacking due to the irresponsibility of the stimuli. He also enumerated the possible etiologies to produce the genetic defect of the polypeptide chain of immunoglobulin as follows.

a. When extremely small amount of a suitable  $m$ -RNA is produced.

b. Appearance of  $m$ -RNA which can not accept the code of normal gammaglobulin production.

c. Appearance of incomplete  $m$ -RNA such as the one which may combine or interfere with the ribosome participating in the production of various polypeptides of gammaglobulin.

His view including his morphological observation has been criticized, nevertheless, his hypothesis is considered worthy to mention.

## B. Heavy chain disease

In 1963, Franklin et al, reported the case with generalized proliferation of lymphatic tissue.<sup>88</sup> Ninety percent of his serum protein was occupied by the beta and gamma globulin fractions. At its highest peak, his serum protein was homogenous and a large amount of his homogenous protein was excreted in the urine. This protein in his blood and urine was non-identical and different from either I or II types which had been customally used for the typing of immunoglobulin.<sup>89-94</sup> However, he reported that it was identical to Fraction B (Fast Component, FC according to the new nomenclature<sup>95</sup>) which was produced from 7-S-gamma globulin under papain treatment.

Following the first case report made by Franklin,<sup>96</sup> Osserman, Takatsuki reported the additional four cases.<sup>97</sup> The common clinical feature of these cases was the high sensitivity to bacterial infection because of the low titers of their various antibodies.

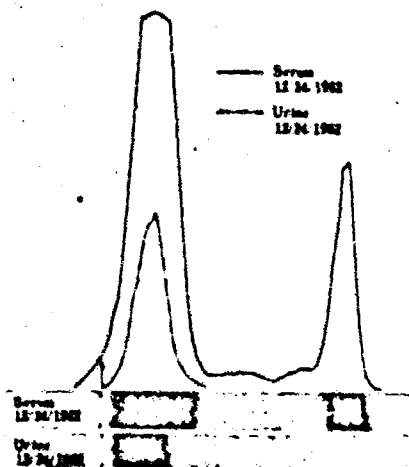


Fig. 2. Paper Electrophoretic Pattern of Serum and Urine of H Chain Disease (from Franklin<sup>96</sup>)

In order to understand the basic principle of the disorder called Heavy chain disease named by Franklin,<sup>96</sup> the polypeptide chain structure of 7-S-gamma globulin must be well apprehended. 7-S-gamma globulin is consisted of four polypeptide chains and a pair of each chain structure is identical each chain is called as H, L chains<sup>98</sup> which is equivalent to A, B chains named by Fleishman, Porter.<sup>99</sup> Its structure model

is illustrated in Fig. 3, 4.<sup>69</sup> in the right portion of Fig. 3, the submitted structure of gamma-2-globulin following being digested by papain. As clearly seen in Fig. 3, L chain is not included in F component at all and Franklin reported lately that the abnormal protein found in blood, urine of heavy chain disease was closely related to F component rather than H chain. As mentioned above, the all five Heavy chain diseases cases except one revealed weak resistances to bacterial infection and had been suffered from pneumonitis repeatedly.

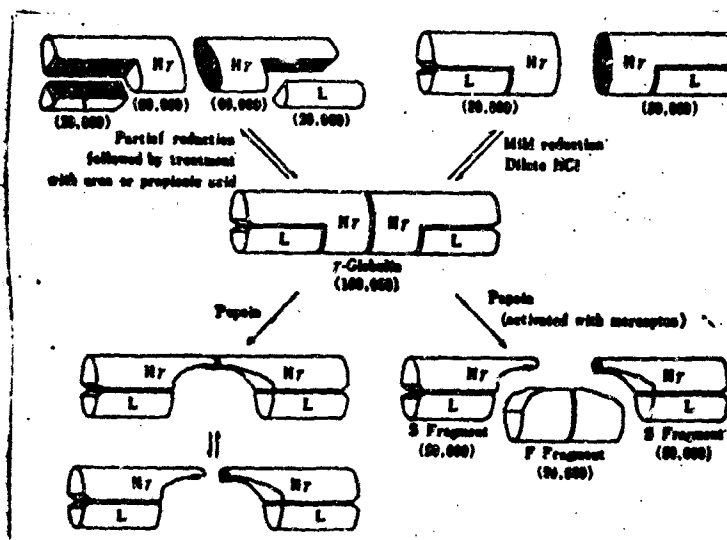


Fig. 3. Chemical and Enzymatic Destruction of alpha-2-globulin (from Echelman<sup>69</sup>)

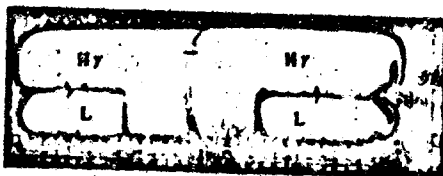


Fig. 4. Structural Model of alpha-2-globulin (from Echelman<sup>69</sup>)

- Comment:
1. Site: Combining site
  2. A number of SS linkages which connects H chains is used to be thought three but Hesono et al corrected it as a single interchain and two intrachains. 100 101

In order to evaluate the productivity of antibody in H chain disease. Franklin investigated the titers of the blood antibodies after these patients were immunized by the potent polyvalent pneumococcal polysaccharide, a booster with diphtheria toxoid and PX 174 bacteriophage.<sup>96</sup> According to his study, only a trace of antibody against the pneumococcal polysaccharide was less than 1% of that seen normally. While a fair amount of antibody was produced to the diphtheria toxoid, the studies of the fractions obtained by the starch block electrophoresis of the patient's serum showed that the antibody resided not with the abnormal protein, but with the normal gamma globulin which normally existed in the serum in trace. Similar attempt to isolate the antibody to IX 174 bacteriophage failed to recover the antibody activity due to the small amount presented. The patient reported by him had Type A blood, but the anti B isoagglutinin titer of his blood was recognized only in the undiluted serum. Judging from the observation made above, it is clear that the abnormal protein in a certain stage of the disease does not hardly possess the antibody activity though its mobility is situated between the beta and gamma globulin in its electrophoretic pattern.

The molecular weight of the abnormal protein mentioned above is 49,000-55,000 (average 51,000) and its precipitation constant is 3.55. It is located between beta and gamma globulin electrophoretically. It is well known that it contains higher amount of carbohydrate than 7-S gamma globulin does.<sup>102</sup> Furthermore, its reaction to heat coagulation is different from Bence Jones protein.

It should be quite interesting to know how H, L chains of immunoglobulin are produced or combined in the gamma globulin producing cell. Bernier labelled the anti-serum both to the Bence Jones protein and to the abnormal protein found in the urine of heavy chain disease which antigen type was different from the former with the double fluorescence method.<sup>103</sup> After the labelling, the reactions of these antiserum to the human spleen and lymph node specimens were observed. According to his study, the cells were classified to those which produce LI Chain only, L II chain only, H chain only, both H and L I chains and H and L II chains. However, the cells producing H chain only were only 5% of the total in his observation of which series did not contain myeloma or Waldenstrom's macroglobulinemia.

In heavy chain disease, it is likely that heavy chain producing cells grow abnormally, as a result, a large amount of H chain was produced, but excreted in urine because of the inavailability of L chain. It is quite significant to

know that the study of Heavy chain disease may settle the discussion about the combining site of gamma globulin.

#### CONCLUSION

Despite the fact that the changes of plasma proteins in several disease have been investigated for several years, its essential understanding is not thoroughly apprehended yet as Williams pointed out. Its change at infection is also without exceptions. The future progress in this particular field is to be expected.

For the sake of economy of space, some of the facets of this problem were not discussed in this brief survey. The authors must leave it to the next opportunity.

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