SOME CONCEPTS OF PLASMA PROTEIN METABOLISM, A.D. 1956

E. Mead Johnson Award Address

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[In presenting the First E. Mead Johnson Award for 1956 to Dr. Gitlin, Dr. Bakwin, President of the Academy, made the following remarks:

"Dr. David Gitlin was born in New York, in 1921. He received the M.D. degree from New York University College of Medicine in 1947. Prior to this he was honored by being the Naumberg Scholar to the University of Puerto Rico in 1940-41, and was awarded the Borden Undergraduate Research Award in Medicine in 1947.

"Dr. Gitlin had his internship at Morrisiana City Hospital; from there he went to Harvard as a Research Fellow in Pediatrics and later served as a Fellow in Medicine; an intern on the Medical Service, an Instructor in Pediatrics, Assistant Physician, and an Associate in Pediatrics, all at the Children's Medical Center and Harvard Medical School.

"Dr. Gitlin is certified by the American Board of Pediatrics and is a Fellow of the American Academy of Pediatrics. During his short career Dr. Gitlin has been an energetic, tenacious, imaginative worker. He has accomplished much in investigation and has published over 30 papers.

"To quote his Chief, Dr. Charles Janeway, 'Dr. Gitlin's researches represent a coherent, progressing series of studies in which, by the use of chemical, immunochemical, and histochemical methods, with good physiologic reasoning, he is gradually elucidating some basic problems of the physiology of human plasma and structural proteins and the derangements in disease.' It is for these works that the Academy's Committee on Awards has selected Dr. Gitlin as the recipient of the First E. Mead Johnson Award for 1956. Dr. Gitlin will honor us by giving a résumé of what he considers to be his important works."

Our sophisticated philosophers would have us believe that man can never be satisfied. A man, so they say, can be rich although he is poor and poor although he is rich. A paradox such as this is an excellent rationalization to soothe one's soul, particularly if one is poor. Today I have been given the privilege of joining the ranks of the very rich, because of the scientific honor associated with this Award. Yet, peculiarly enough, this is not where the greatest satisfaction lies. This occasion has yielded rewards that transcend the pride of the moment. It has given me the opportunity to]
appreciate more fully my friends and colleagues; your good will and sincere good wishes have given me more pleasure than the Award would have otherwise. And the occasion serves also, in some small way, to justify the faith and teachings of the dedicated men who guide my studies. Among these: Dr. Colin MacLeod and his associates who took me in as a medical student and tried to instill that most important element of research, freedom of thought; Dr. Louis Diamond, Dr. Stewart Clifford and Dr. Sydney Gellis who tried so valiantly to teach me pediatrics and that life is not necessarily as grim as it sometimes seems; Dr. Walter Hughes and Dr. J. L. Oncley who gave so generously and unselfishly of their knowledge of protein chemistry; Dr. Sidney Farber and Dr. John Craig who have given of their sincere cooperation on innumerable occasions.

To Dr. Charles A. Janeway, however, remained the most severe task of all—the integration of these teachings and the formation of an individual. He has been my guide, my critic, my advisor, and my teacher, in personal matters as well as scientific, and to him I owe my pediatric career. In all candidness, the work for which this Award has been given was due to his efforts as much as to mine.

In accepting this Award, then, I accept it in the names of these men and our collaborators and friends of many different institutions.

I have been asked to select highlights from our work upon which this Award has been based. Because of the many ramifications of our studies and depending upon the work of so many other investigators, this is as heartless as asking a student to condense an encyclopedia to an essay. And how to select the highlights when we are not sure just what they are? What may be the important factors to us today may be the nonsense of tomorrow. However, as the title states, we should like to discuss some simple aspects of human plasma protein metabolism.

Just as man's nature and emotions are in a continuous state of flux, so is his body chemistry; the plasma proteins share in this general unrest. The individual plasma proteins are synthesized or born at certain rates and they are catabolized or die at certain rates. The amount of a specific plasma protein present in the body at any given time, therefore, represents the brief existence of a group of its molecules between the times of their synthesis and degradation. For the amount of this protein in the body to remain constant, then, the rate of synthesis must equal the rate of loss of that protein from the body whether by catabolism or by any other route of loss.

When we measure concentrations of a particular plasma protein, we are localizing a group of its molecules in a given place at a given time. The individual protein molecules, however, are in constant activity. A molecule is synthesized, eventually released into the circulation and remains intact as an individual molecule for a variable period of time. Theoretically, a molecule may be catabolized immediately after synthesis or it may remain in the body fluids for months. Taken as a whole, however, a given proportion or fraction of the total body pool is catabolized per unit time. This fraction of the total body pool may be termed the fractional rate of catabolism while the actual amount catabolized per unit of time, expressed for example in grams per day, is the rate of catabolism.

Our studies until now have been concerned with the peregrinations of some of the plasma protein molecules. It is possible, as you know, to label a group of protein molecules in a variety of ways—with radioactive iodine, sulfur, carbon, or even with certain dyes. When a trace amount of a plasma protein is injected into the vascular system, the plasma concentration of the injected protein ordinarily follows a definite curve.1-4 Initially, the concentration falls relatively rapidly and then, after a period of some days, the decline in concentration follows a logarithmic or exponential function. The rate of exponential decline is a measure of the catabolism of the tracer pro-
tein being studied. The initial rapid fall in concentration which occurs after distribution within the blood is due to the diffusion of the protein from the vascular system.

Let us take a specific example. In Figure 1 is plotted the plasma-disappearance curve for radio-iodinated human serum albumin in a normal child. In this instance, during the logarithmic phase, the concentration of labeled albumin fell at a rate such that 50%, or half, of a given number of labeled albumin molecules was catabolized about every 15 days. The latter value may be termed the half-life of albumin due to catabolism and, when applied to the child's endogenously synthesized albumin, states that half of the child's total body albumin was catabolized every 15 days.

Note that this particular curve was constructed by plotting plasma concentration versus time. We can demonstrate that the plasma volume was constant during the course of the study. If the plasma concentrations are multiplied by the plasma volume (a constant value), this does not change the shape of the curve, but now the ordinates indicate the total amount of labeled albumin in the vascular or plasma compartment and the value at zero time represents the total amount of tracer given to the child. Extrapolating the catabolism portion of the plasma-disappearance curve to zero time, one can obtain that fraction of the total body pool of the tracer that is in the plasma during the steady state, or after the tracer was distributed throughout the body fluids.

Thus, from the catabolism curve of radio-iodinated albumin, only 50% of the labeled albumin can be accounted for in the plasma compartment after the protein has been distributed throughout the body. It is apparent that approximately 50% of the injected tracer left the vascular system within the first 5 to 7 days. If endogenous albumin behaves as does radio-iodinated albumin, then in the steady state, about half of an individual's total body albumin should be present extravascularly.

In the presence of edema or in the presence of very rapid catabolism of the plasma protein being studied, this type of graphic analysis of plasma-disappearance curves is not applicable to the body pool of endogenous plasma protein. Under these circumstances, the specific activity of the tracer in the various body fluids, i.e., amount of tracer per unit amount of unlabeled
analogous protein, would not be equal, a condition essential to this method of analysis.\textsuperscript{5,6} There are a number of other, but more difficult, ways of estimating that fraction of the total body content of a specific plasma protein that is in the plasma, and hence that fraction present extravascularly\textsuperscript{11,5} but it is not possible to discuss them at this time.

The rapidly falling portion of the radioiodinated-albumin curve, then, is attributable to two factors: 1) migration of the tracer from the vascular system, and 2) catabolism. The rate of extravascular diffusion is much greater than the rate of catabolism.

From the definition of half-life and from the mathematical characteristics of the phase of catabolism the following equation can be derived:

\[
\text{Half-life due to catabolism} = \frac{0.693 \times \text{Total amount of albumin in the body (T)}}{\text{Amount of albumin catabolized per day (A_c)}}
\]

The catabolic half-life for albumin has al-
ready been estimated as 15 days in the example given. Then to determine the rate of catabolism, \( A_r \), it is necessary to know the total amount of albumin in the body, \( T_A \). The plasma concentration of albumin in this child was 3.5 gm/100 ml and the plasma volume was 1500 ml; thus, the vascular system contained 
\[
\frac{3.5 \text{ gm}}{100 \text{ ml}} \times 1500 \text{ ml} = 52.5 \text{ gm}.
\]
However, the vascular system contains about 50% of the total body albumin in this case and hence the total body albumin can be calculated:
\[
52.5 \text{ gm} = 0.50 T_A \text{ or } T_A = 105 \text{ gm}.
\]
The rate of catabolism, \( A_r = \frac{0.693 \times 105 \text{ gm}}{15 \text{ days}} \) = 4.85 gm/day.

Since the child was in a steady state with respect to albumin metabolism, that is, the total concentration of albumin was neither rising nor falling, then the rate of synthesis must have been equal to the rate of catabolism. Therefore, the rate of synthesis of albumin in this instance was also 4.85 gm/day. The behavior of newly synthesized albumin entering the vascular system directly or indirectly from the sites of synthesis would be that already described by the injection of iodinated albumin into the vascular system (Fig. 3).

We have pointed out that molecules of the various plasma proteins continuously diffuse from the vascular system into the interstitial fluids. That the extravascular plasma proteins return to the vascular system also appears to be true. To test this experimentally, normal rabbits were given a concentrated solution of rabbit antipneumococcal antibodies intravenously (Fig. 4). This was a passive transfer; the recipient rabbits were not synthesizing these specific antibodies. As was to be expected, initially there was a rapid fall in the plasma concentration of antibody followed by the characteristic exponential decline. During the latter phase, when the antibody was in a steady state with respect to its relative distribution in the body fluids, most of the antibody present in the plasma was removed by exchange transfusion or by a reaction with specific antigen. The plasma
concentration of antibody fell precipitously, then rose rapidly from the low values attained and then resumed the phase of logarithmic fall. The rapid rise was due to the shift of antibody from the extravascular compartment. Thus, extravascular plasma protein is in dynamic equilibrium with intravascular plasma protein and a decrease in the mass of a specific plasma protein in one compartment results in the movement of plasma protein to that compartment until a steady state is once again attained. The clinical importance of this large extravascular reservoir of preformed plasma protein in homeostasis should not be underestimated. Thus, after an acute hemorrhage, for example, extravascular plasma protein would rapidly re-enter the circulation and maintain oncotic pressure. Actually, the extravascular plasma protein is not truly a reservoir in the sense of a stagnant reserve, but instead represents simply that plasma protein which is outside the vascular compartment at any given moment. The individual protein molecules are in constant motion, going into or leaving the extravascular areas.

From what has been discussed thus far and from additional evidence obtained in animals, it will be noted that the catabolism of a given plasma protein is a first-order chemical reaction. Under these circumstances: 1) the half-life of a given plasma protein due to catabolism is independent of the total body pool or plasma concentration; e.g., the normal half-life of albumin would be about 15 days, whether the plasma concentration were 3.5 gm or 0.35 gm/100 ml, and 2) the rate of catabolism of a given plasma protein in terms of amount catabo-
lized per day is dependent upon the total body pool of that protein; e.g., at a plasma concentration of 1000 mg/100 ml, the rate of catabolism would be 10 times that when the concentration is 100 mg/100 ml, provided, of course, that the body distribution of that protein in both instances is identical. These facts are not always clearly seen in clinical investigation, however, since alteration of the body pool or plasma concentration of a protein is frequently either the result of a primary change or is accompanied by a compensatory change in the catabolic half-life of the protein.

The same metabolic and mathematical considerations discussed for albumin apply equally well to the metabolism of γ-globulin, fibrinogen, iron-binding globulin and other plasma proteins. But the inadequacy of a given tracer or label for the plasma proteins may make certain quantitative data subject to considerable doubt although qualitatively, the labeled protein may behave like its unlabeled analogue. This seems to be the case for γ-globulin.

Unlabeled γ-globulin, in the form of whole plasma, injected intravenously into children with congenital agammaglobulinemia has a similar rapid initial fall in concentration followed by a phase of slower exponential decline. No exogenous label is used and determinations are made by immunochemical methods. The initial part of the curve, as with albumin, is associated with the appearance of γ-globulin in the interstitial fluid of the connective tissues throughout the body. The half-life of the unaltered γ-globulin in these patients, however, is 30 to 60 days (Fig. 5). Radiiodinated γ-globulin prepared from concentrated purified γ-globulin has a half-life of about 20 days in the same patients (Fig. 6). The difference in half-lives would give proportionate differences in the calculated rates of catabolism. The same preparation of concentrated γ-globulin used for the iodination procedure when given intramuscularly and unlabeled has a half-life of 30 to 60 days after the steady state has been reached. The differences in half-lives are attributable either to the iodination procedure or to the atoms of iodine on the γ-globulin molecule.

In addition, there is no method presently available which will accurately estimate the concentration of γ-globulin in plasma or other body fluids. These difficulties leave the quantitative aspects of γ-globulin metabolism with much to be desired. However, for practical purposes, even calcula-
Consider the simple problem of deciding how much γ-globulin would be necessary to raise the plasma concentration in a child by 100 mg/100 ml. As the plasma volume is roughly 5% of the body weight, there would be 50 ml of plasma per kilogram of body weight. Fifty milliliters of plasma per kilogram multiplied by 100 mg of γ-globulin per 100 ml of plasma, the increment desired, is 50 mg of γ-globulin/kg; this is the amount needed for the plasma component. But only half of the γ-globulin given will remain in the vascular system and hence twice this amount or 100 mg of γ-globulin/kg would raise the concentration in the vicinity of 100 mg/100 ml. With a half-life of roughly 30 days, the increment in concentration would fall to 50 mg/100 ml at the end of this time; an additional injection of 100 mg of γ-globulin/kg of body weight would then raise the increment to a total of 150 mg/100 ml. At the end of 30 days the concentration would be about 75 mg/100 ml. γ-Globulin administered intramuscularly (Fig. 7) readily reaches the vascular system; in fact, after the steady state has been reached, the concentration achieved in the plasma via the intramuscular route is essentially the same as that attained via the intravenous route.

The total amount of a specific plasma protein in the body is dependent upon its rate of synthesis and its rate of catabolism. It is not reasonable, then, to attempt to predict the metabolic mechanisms involved in any given protein or group of proteins, and it is the mechanisms involved that dictate the type of replacement therapy. For example, patients with agammaglobulinemia and patients with the nephrotic syndrome manifest very low concentrations of γ-globulin in the body; yet, in each instance, the mechanism is quite different and replacement therapy is effective in one, but impractical and ineffective in the other.

In agammaglobulinemia the defect is one in synthesis. A patient with either the congenital or acquired form of this disease has few, if any, plasma cells; these cells are the sites of synthesis of antibodies or γ-globulin (Fig. 8) in addition, perhaps, to several other plasma proteins. This state of affairs is reflected in the normal or less than normal fractional rate of catabolism of administered γ-globulin in these patients. To obtain insignificant concentrations of γ-globulin in the face of no increase in the fractional rate of catabolism, synthesis would have to be insignificant; we have already discussed how the actual rate of synthesis may be calculated from such data.

In children with the fully developed nephrotic syndrome, on the other hand, tracer studies reveal that the low concen-
trations of albumin, \(\gamma\)-globulin, and iron-binding globulin found in this disease are due to a greatly increased fractional rate of catabolism in combination with severe urinary losses (Table I). The over-all loss of albumin, for example, is so great that these children lose at least half of the total body plasma albumin every 12 hours. The rate of synthesis of these proteins in this disease is not greatly increased. It is interesting that in a child with a mild nephrotic syndrome, i.e., with minimal or no ascites and minimal edema, the low plasma protein concentrations may be due either to severe urinary losses or to an increased fractional rate of catabolism, but not both.
### TABLE I

THE TURNOVER OF CERTAIN PLASMA PROTEINS IN THE NEPHROTIC SYNDROME

<table>
<thead>
<tr>
<th>Patient</th>
<th>Stage of Disease</th>
<th>Per Cent of Body Pool Turned Over Per Day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Catabolism</td>
</tr>
<tr>
<td>Albumin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R.S.B.</td>
<td>Latent</td>
<td>4.0</td>
</tr>
<tr>
<td>A.B.</td>
<td>Mild (minimal edema)</td>
<td>25.2</td>
</tr>
<tr>
<td>E.T.</td>
<td></td>
<td>7.9</td>
</tr>
<tr>
<td>K.S.</td>
<td>Severe (anasarca and ascites)</td>
<td>43.4</td>
</tr>
<tr>
<td>S.L.</td>
<td></td>
<td>33.1</td>
</tr>
<tr>
<td>D.W.</td>
<td></td>
<td>16.2</td>
</tr>
<tr>
<td>Normal (average)</td>
<td></td>
<td>5.0</td>
</tr>
<tr>
<td>Gamma Globulin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R.S.B.</td>
<td>Latent</td>
<td>2.8</td>
</tr>
<tr>
<td>A.B.</td>
<td>Mild</td>
<td>11.3</td>
</tr>
<tr>
<td>E.T.</td>
<td></td>
<td>8.1</td>
</tr>
<tr>
<td>K.S.</td>
<td>Severe</td>
<td>10.4</td>
</tr>
<tr>
<td>S.L.</td>
<td></td>
<td>7.9</td>
</tr>
<tr>
<td>D.W.</td>
<td></td>
<td>15.1</td>
</tr>
<tr>
<td>Normal (average)</td>
<td></td>
<td>3.5</td>
</tr>
<tr>
<td>Iron-binding Globulin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A.B.</td>
<td>Mild</td>
<td>21.9</td>
</tr>
<tr>
<td>E.T.</td>
<td></td>
<td>18.2</td>
</tr>
<tr>
<td>S.L.</td>
<td>Severe</td>
<td>21.3</td>
</tr>
<tr>
<td>Normal</td>
<td></td>
<td>5.0</td>
</tr>
</tbody>
</table>

as in the child with severe nephrotic syndrome. In addition, careful examination of the data will reveal that the degree of proteinuria as measured by amount excreted per day may bear little or no relationship to the severity of the disease in any given instance. Thus, in Table I, patient E.T. with mild disease and patient S.L. with a severe nephrotic syndrome both excreted about 5 gm of albumin in the urine per day. But in the case of S.L., there was the added difficulty of a greatly increased fractional rate of catabolism. As the rate of synthesis of albumin was about the same in these two patients, patient S.L. could not make up the loss as well as E.T. and had more severe manifestations of hypalbuminemia. Again, patients K.S. and D.W. excreted but 2 gm of albumin per day and the manifestations of the disease were as severe as those of S.L. and much more severe than in A.B. who also excreted 2 gm of albumin per day. Thus, in the presence of two variables, 1) the fractional rate of catabolism of albumin and 2) the renal clearance of albumin, it is necessary to know both values to determine their relative influence on the production of a third factor—symptoms attributable to hypalbuminemia, viz., anasarca and ascites.

One might argue that the plasma concentrations of γ-globulin in nephrosis are higher than those ordinarily found in agammaglobulinemia. This is true for the con-
Fig. 9. A simplified diagram of the metabolism of β-lipoprotein in normal individuals and in children with the nephrotic syndrome.

Fig. 10. Sections of biopsies from a child with afibrinogenemia stained for fibrinogen by the fluorescent-antibody method. A + B: Absence of fibrinogen from the connective tissue (arrows) of skin (A) and muscle (B). C + D: Presence of fibrinogen in the connective tissue of skin (C) and muscle (D) biopsies taken 24 hours after the intravenous infusion of fibrinogen. (All ×210.)
genital, but not the acquired, form of the latter disease, where concentrations of \( \gamma \)-globulin between 50 and 100 mg/100 ml are not uncommon. That nephrosis and agammaglobulinemia are distinguishable clinically, cannot be argued, but there are individuals with an isolated finding of severe hypogammaglobulinemia on the basis of increased catabolism, just as in nephrosis. These are taken simply as examples. Another example of similar nature is hypoaalbuminemia as a result of failure in synthesis in contrast to idiopathic hypoaalbuminemia on the basis of an increased fractional rate of catabolism. There are many other examples.

Similarly, in cases where a high plasma concentration of a specific protein may exist, it is not possible to predict in advance whether there is an increased rate of synthesis or a decreased fractional rate of catabolism or loss. In the nephrotic syndrome, for example, all of these factors play a part in the production of the hypercholesterolemia and hyperlipoproteinemia found in this disease. In the normal individual, low-density \( \beta \)-lipoproteins, containing relatively large amount of lipid, are synthesized and released into the plasma; these are in part catabolized directly, but for the most part are converted into high-density \( \beta \)-lipoproteins, or \( \beta \)-lipoproteins with less lipid, albumin being necessary in the mechanism for the removal of released fatty acids (Fig. 9). The low-density \( \beta \)-lipoproteins are catabolized at a definite rate. In the nephrotic

![Fig. 11. Sections of lungs from children succumbing to “hyaline-membrane disease,” stained for fibrin by the fluorescent-antibody method. The white areas, indicating fibrin, clearly delineate the membranes in the alveolar ducts. (x210.)](http://pediatrics.aappublications.org/)

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Fig. 12. Sections of tissue from patients with “collagen diseases” stained for fibrin (white areas) by the fluorescent-antibody method. A. Fibrin in a rheumatoid nodule. (×100.) B. Fibrin in the proliferating intima of a renal blood vessel in a patient with polyarteritis. (×210.) C. Fibrin in the glomerulus of a child with lupus erythematosus disseminatus. (×210.) D. Fibrin in myocardial connective tissue and around necrotic muscle fibers in a child with lupus erythematosus disseminatus. (×210.)

syndrome, there is a greatly increased synthesis of low-density or lipid-rich \( \beta \)-lipoproteins as well as a decreased rate of conversion to the high-density \( \beta \)-lipoproteins.\(^5\) The poor rate of conversion is apparently due in part to the very low concentration of albumin in the plasma encountered in this disease.\(^2\) Consequently, as the result of these factors, there is an accumulation of the low-density \( \beta \)-lipoproteins in the plasma with the accompanying lipids.

This study of the metabolism of the plasma proteins has sometimes led us into strange paths. In a study of the distribution of fibrinogen, it was found that this protein also diffuses from the vascular system.\(^2\) It, too, is normally present in the interstitial fluids.\(^3\) In a patient with a fibrinogenemia, fibrinogen given intravenously can be detected in the connective tissues within 24 hours (Fig. 10). A study of the nature of the hyaline membrane found in certain newborn infants succumbing to asphyxia\(^2\) revealed that this membrane is made up principally of fibrin, as demonstrated by the fluorescent antibody method (Fig. 11). As there is little or no fibrin demonstrable in human amniotic fluid, the fibrin in the hyaline membrane must have come from the infant. Fibrin cannot traverse the capillary walls, but fibrinogen can. It would appear from the evidence obtained that the hyaline membrane is formed as the result of an effusion from the pulmonary capillaries, the fibrinogen in the effusion being then converted to fibrin. Because amniotic fluid con-
tains thromboplastic materials, its presence in the lung at the time of the effusion would enhance the conversion of fibrinogen to fibrin. The cause of the effusion, however, has not yet been elucidated.28,29 Amniotic fluid in the lung apparently does not induce such effusions. We are inclined to the opinion that this effusion may be accounted for on the basis of left ventricular failure, possibly due to sudden increase in peripheral resistance encountered when the umbilical cord is tied off.

A study of the nature of fibrinoid in collagen diseases29 has led us to the conclusion that the bulk of the fibrinoid material found in these lesions is also fibrin (Fig. 12). The lesions in these diseases would appear to be inflammatory in nature rather than degenerative.

ACKNOWLEDGMENTS

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