

## CONGENITAL ABNORMALITIES IN PROTEIN SYNTHESIS

By David Gitlin, M.D., and Charles A. Janeway, M.D.

*Department of Pediatrics, Harvard Medical School, and the Children's Medical Center, Boston*

Most, if not all, of congenital metabolic disease may be attributed to abnormalities in protein synthesis. An error in protein synthesis can result in at least two basic types of disturbance—an actual deficit of a specific protein, or the production of abnormal forms of that protein. Because of the wide variety of functions of this class of substances, the result may manifest itself as a disturbance in the metabolism of fats, carbohydrates or amino acids, or a disturbance of protein interactions.

While the clinical manifestations of some disorders may be related *directly* to the deficit of a specific protein, in others symptoms are related *indirectly* to a specific protein deficiency (Fig. 1). This is particularly true in those instances where the protein, which is deficient, acts as an enzyme in intermediary metabolism, and the symptoms of disease may be due either to an accumulation or a deficit of specific metabolites or electrolytes. In still other instances, clinical disease is related not to the absence of a normal protein but to the presence of an abnormal protein which does not function in the same way as its normal analogue.

It is not the purpose of this paper to discuss all the inborn errors of protein metabolism, but to give a brief account of some examples to illustrate the fundamental importance of protein synthesis to human health.

## CONGENITAL AFIBRINOGENEMIA

Afibrinogenemia, a hemorrhagic diathesis associated with the absence of fibrinogen in the blood and a clotting time of infinity, was recognized as early as 1920.<sup>1</sup> Since that time a number of children with this disorder have been described and, in a review by Diamond, Wolff and Borges, 19 cases, including 3 observed by these

authors, are reported.<sup>2</sup> The disorder has appeared in either sex, there is a high incidence of consanguinity among the parents, and hemorrhagic episodes following trauma often begin early in life, recurring at irregular intervals. The infusion of sufficient fibrinogen in these children immediately restores the clotting time to normal.

An abnormally low concentration of a specific protein, however, does not of itself indicate an error in the synthesis of that protein. Any of *three* possible mechanisms might account for a protein deficit at a particular site: 1) inadequate synthesis of the protein; 2) abnormal distribution of the protein within the body; or 3) an abnormally rapid turnover of the protein, due to an increased rate of catabolism or of utilization of the protein in its physiologic role, or to loss from the body. That the paucity of circulating fibrinogen in patients with congenital afibrinogenemia was attributable to insufficient synthesis was shown by transfusing two of these children with fibrinogen as Fraction I derived from normal human plasma.<sup>3</sup> The half-life of the transfused fibrinogen was found to be approximately 4 days or about normal; thus the afibrinogenemia could not be due to an abnormally rapid turnover of fibrinogen.

Using immunochemical methods which are so sensitive that they can be used for detection of traces of proteins in mixtures, the plasma concentration of fibrinogen in the steady state in these children was found to be about 1 mg/100 ml (normal, 250 to 400 mg/100 ml) and that even this fibrinogen was distributed in the body proportionately as in the normal individual. Thus, congenital afibrinogenemia must be due to inadequate synthesis of fibrinogen.

It will be noted that despite the use of the term afibrinogenemia, very small

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ADDRESS: (D.G.) 300 Longwood Avenue, Boston 15, Massachusetts.



rinogenemia, no morphologic evidence to account for defective fibrinogen synthesis has been found, in congenital agammaglobulinemia inadequate  $\gamma$ -globulin synthesis is associated with a disturbance of the architecture of the lymphoid follicles and failure to form plasma cells after antigenic stimulation.<sup>9, 10</sup> Since lymphoid tissues and specifically plasma cells appear to be the primary source of antibodies and presumably of  $\gamma$ -globulin,<sup>8</sup> the deficiency of plasma cells would appear to be responsible for the paucity of  $\gamma$ -globulins.

Not only are  $\gamma$ -globulins almost absent in this disorder, but there is also a deficiency of at least two  $\beta$ -globulins which are immunochemically unrelated to the  $\gamma$ -globulin.<sup>11</sup> Whether these proteins are also synthesized by plasma cells is not known, but it is interesting to speculate that they are. In any event, congenital agammaglobulinemia represents a gross abnormality in protein synthesis in which specific whole cells are not formed. Thus, while the major symptoms of the disorder are directly related to the deficiency of a specific group of proteins (the  $\gamma$ -globulins), the deficiency of the latter is but a reflection of more fundamental errors in protein synthesis resulting in the absence of plasma cells.

#### HEPATOLENTICULAR DEGENERATION

Wilson's disease, or hepatolenticular degeneration, is associated with abnormal accumulations of copper in tissues, particularly in the brain, liver and kidneys.<sup>12, 13</sup> From the data available, it would appear that the clinical manifestations of the disease can be attributed to the toxicity of the accumulated copper in these major organs; an induced loss of copper in these patients is associated with some clinical improvement.<sup>14</sup> Also associated with this disease is a deficiency of ceruloplasmin.<sup>15</sup> Ceruloplasmin is a blue-colored plasma protein with a molecular weight of about 151,000 which contains 8 atoms of copper per molecule.<sup>16</sup> It is capable of functioning as a weak oxidase, its capacity to oxidize ascorbic acid

or phenylenediamine often being used as a method for its determination.

Normally, over 90% of the copper in plasma is found in ceruloplasmin. In Wilson's disease the non-ceruloplasmin plasma copper is markedly elevated. Scheinberg has shown that the half-life of ceruloplasmin in Wilson's disease is about 4 days, and calculations indicated that the deficiency of ceruloplasmin was probably due to inadequate synthesis.<sup>17</sup> By labeling ceruloplasmin with I-131, it has been shown that the half-life of ceruloplasmin in patients with neurologic disease other than hepatolenticular degeneration is about 5 to 7 days, thus confirming Scheinberg's calculations.<sup>18</sup>

The exact relationship between ceruloplasmin and copper metabolism in general is not known. That the deficiency of ceruloplasmin may not be the cause of Wilson's disease is indicated by the fact that prolonged administration of ceruloplasmin has not effected an amelioration of the disease. Chalmers *et al.*<sup>19</sup> have presented evidence suggesting that the abnormal copper metabolism is due to a general error in protein metabolism. This is supported by recent findings in mice indicating that the accumulation of copper in Wilson's disease is not due to an increased absorption of copper as had been thought, but rather to an inadequate excretion of copper via the gastrointestinal tract.<sup>20</sup> Should this be confirmed, Wilson's disease would be an instance where defective synthesis of a specific protein, ceruloplasmin, is indirectly associated with the metabolism of a cation, copper, both being due to a more fundamental defect in protein metabolism resulting in the failure of copper excretion.

#### DEFICIENCIES IN FACTORS CONVERTING FIBRINOGEN TO FIBRIN

Deficiencies of various protein components of the coagulation system, other than fibrinogen, represent examples where the paucity of one specific protein may inhibit the functioning of several other proteins to

produce clinical manifestations of disease. The latest, but not final, opinion of experts on coagulation is that thromboplastin, calcium and two plasma factors (one of which is stable and the other unstable) are necessary for the conversion of prothrombin to thrombin; thrombin acts as an enzyme to convert the substrate fibrinogen to fibrin in the final phase of the coagulation chain reaction. To obtain thromboplastin, it is necessary for plasma to contain antihemophilic globulin, thromboplastin component and thromboplastin antecedent, as well as one or more factors derived from platelets. Congenital deficiencies of each of the plasma protein components mentioned have been described. In each instance, the rate and extent of thrombin formation is decreased and the conversion of fibrinogen to fibrin is ultimately inhibited with resulting hemorrhagic tendencies.

#### GALACTOSEMIA

Galactosemia is a congenital disorder in the metabolism of a specific carbohydrate, galactose.<sup>21</sup> Galactose normally enters the metabolic pathways of glucose by being first phosphorylated to form galactose-1-PO<sub>4</sub>, which is then converted to glucose-1-PO<sub>4</sub>.<sup>22</sup> The outstanding work of Kalckar and his colleagues<sup>23</sup> has demonstrated that the metabolic defect in galactosemia is a deficiency of the enzyme necessary to convert galactose-1-PO<sub>4</sub> to glucose-1-PO<sub>4</sub>; this enzyme is known as P-Gal transferase.

It is now well known that removal of foods containing galactose (e.g., as glycosides) from the diet of children with galactosemia will ameliorate the condition of these patients if done early enough. The relation between the abnormal galactose accumulation and the clinical manifestations of the disease, such as hepatomegaly, splenomegaly and nuclear cataracts, seems well founded. In galactosemia, then, inability to synthesize adequately a specific protein enzyme results in a disturbance of carbohydrate metabolism which in turn produces the clinical disease which we recognize in patients.

Without going into further detail of the functions of proteins as enzymes, similar examples could be cited where deficits in the formation of specific enzymes may interfere with the metabolism of specific amino acids, as a result of which the accumulation of normal or abnormal metabolites or the lack of intermediates for further synthetic reactions gives rise to the functional or pathologic changes which characterize the disease.

#### ABNORMAL HEMOGLOBINS

After the remarkable discoveries by Pauling, Itano and others, the study of abnormal hemoglobins has proceeded rapidly. Pauling<sup>24</sup> was able to demonstrate that the erythrocytes of patients with sickle cell anemia are characterized by the presence of a type of hemoglobin, now designated as hemoglobin S, identified by an abnormal electrophoretic mobility. It was then shown that the amount of this hemoglobin present in the erythrocytes was controlled by heredity; the cells of those individuals with the sickle cell trait (heterozygotes) contained less hemoglobin S than those with manifest sickle cell disease (homozygotes).<sup>25</sup>

Scheinberg<sup>26</sup> demonstrated that the unusual electrophoretic behavior of hemoglobin S was attributable to a decreased negative charge due to the absence of two carboxyl groups. It was then shown that this hemoglobin differs from the normal only in the substitution of two molecules of the amino acid valine for two molecules of glutamic acid.<sup>27</sup> Since normal hemoglobin is made up of 577 molecules of amino acid, of which 38 are glutamic acid, it is striking that this slight difference in molecular composition should make such a radical difference in molecular behavior. Hemoglobin S is very sensitive to differences in pH and with a slight lowering of pH the molecules rearrange themselves in the form of tactoids; this is reflected by the sickling of the erythrocytes which contain them.

Under appropriate conditions, an individual with sickle cell disease having from

60 to 90% of his hemoglobin as hemoglobin S is subject to hemolytic crises. Greenberg and Kass<sup>28</sup> have shown that these can be stopped with massive infusions of sodium bicarbonate.

Study of other abnormal hemoglobins is progressing; this group of disorders provides an excellent opportunity to study abnormal protein synthesis and its genetic control.

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