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REVIEW OF SOME OF THE MORE RECENT ADVANCES IN THE STUDY OF BLOOD DISEASES[1]

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PROGRESS in hematology, as in any other science, depends to a great extent upon new and accurate methods of observation. As has been emphasized by Sir Humphry Rolleston,[2] probably the two most important steps which led to a great increase in our knowledge of blood diseases were the introduction of the microscope, and especially the compound, achromatic form by G. D. Amici a hundred years ago, and the development of the modern methods of staining blood by Paul Ehrlich, which he began in 1877.

The entire field of medicine from a scientific standpoint has progressed more rapidly during the past 50 years than it has at any other time in the history of the world. New, valuable and accurate information which has an important bearing on the cause and cure of disease has accumulated so rapidly that those who teach the practice of medicine and are interested in problems of medical research find that it requires a great effort on their part to keep abreast with the advances which are being made, even when their interests are largely restricted to a specialized field.

The science of hematology illustrates as well as any other branch of medicine that these statements are true, for during the past decade many new and fundamental facts have been discovered which have an important bearing upon the etiology and the treatment of certain blood diseases.

As it will be necessary obviously to limit this discussion, it is essential that my remarks be confined to

[1] Address of the vice-president and chairman of the Section of the Medical Sciences, delivered before the General Session, American Association for the Advancement of Science, Pittsburgh, December 27, 1934.

[2] Sir Humphry Rolleston, *Proc. of the Royal Soc. of Med.*, 27: 31–48, July, 1934.

a few outstanding achievements in this field which have been developed recently on a sound scientific basis. I propose to discuss only three phases of hematology in which recent and important contributions have been made to our knowledge. They are: (1) The etiology and treatment of pernicious anemia; (2) the iron-deficiency anemias and their treatment; and (3) the etiology of agranulocytosis.

Pernicious anemia is a disease which was first described by Thomas Addison in 1849, 85 years ago. Until 1926, the following would have been a correct and concise definition of the condition: "It is a disease of unknown causation, most frequently occurring at middle life or later, usually characterized by a marked reduction in the red blood cells and hemoglobin of the peripheral blood, always associated with an absence of hydrochloric acid in the gastric secretions, and frequently complicated by degenerative changes in the nervous system." It was also necessary to add to this definition that the disease progressed, usually with spontaneous remissions, to a fatal termination within 2 or 3 years from the earliest appearance of symptoms. Before that time many types of treatment had been advocated, but with the possible exception of blood transfusions which had only a very transient beneficial effect, it could not be proven that any form of therapy prolonged the life of a given patient a single day.

Interest in the modern treatment of pernicious anemia dates from the experimental work of George H. Whipple, who in 1925[3] demonstrated that the blood of dogs, made anemic by repeated hemorrhages, would return to normal more rapidly if liver was added to the diet. It is undoubtedly true that other investigators had previously used liver in the treatment of various types of anemia, but it was the work of Whipple and later of Minot and Murphy[4] which brought this form of therapy to the attention of the general medical profession. The latter observers proved conclusively that the feeding of 240 grams of calf's liver daily would invariably induce a remission in a patient with pernicious anemia, which usually began within 3 to 6 days after the treatment was instituted. The resultant increase in red blood cells was usually at the rate of 400,000 to 500,000 cells per cu. mm. per week until the normal level of 4.5 to 5 million per cu. mm. was reached. Simultaneously with this improvement in the blood condition, there was a remarkable disappearance of all the patient's symptoms, and, unless some complication existed, there was a complete return to a state of normal health. Moreover, it has been demonstrated that this condition can be maintained apparently for an indefinite period of time, provided an adequate amount of liver is consumed. Subsequent developments in liver therapy have been the production of a concentrated soluble liver extract for oral use by Minot and Cohn[5] and similar potent products for intramuscular use by Gansslen[6] and intravenous use by Castle and Taylor.[7] These facts which have just been related demonstrate that the liver contains an unidentified substance which will cause the blood of a patient with pernicious anemia to return to normal if it is administered in appropriate amounts. Additional investigation has shown that the anemia of pernicious anemia exists primarily because there is a delay in the development of the red blood cells in the bone marrow.[8] As a result they can not be released to the peripheral blood in sufficient numbers to maintain the red blood cell count at a normal level. The failure of the red blood cells to develop normally has been termed a "maturation arrest." It can be stated definitely, therefore, that this form of anemia is due to defective blood formation.

Within recent years Castle and his associates[9] have completed and published a brilliant series of investigations dealing with the relation of the stomach to the cause of pernicious anemia, which has given more information concerning the etiology of this condition than any other contribution. For many years it was thought that a defect of the stomach had some causal relationship to pernicious anemia, but the exact nature of this association was unknown until Castle reported his recent studies. That some disorder of the stomach played a rôle in the etiology of pernicious anemia was suggested by the almost constant presence of gastric symptoms in patients with the disease, the uniform lack of hydrochloric acid in their gastric secretions and the rare but definite observation that complete removal of the stomach in human beings was followed by changes in the blood which were identical with those of pernicious anemia. In brief, it was demonstrated by Castle that when gastric juice which is obtained from normal human beings is incubated with uncooked Hamburg steak, a substance is elaborated which has the same action and is as effective as liver in controlling pernicious anemia. These experiments, which have been adequately controlled and confirmed, indicate clearly that there is some unidentified substance (the intrinsic factor of Castle) in nor-

[3] G. H. Whipple and F. S. Robscheit-Robbins, *Am. Jour. Physiology*, 72: 408, May, 1925.

[4] G. R. Minot and W. P. Murphy, *Jour. Am. Med. Assoc.*, 87: 470, Aug., 1926.

[5] E. J. Cohn, G. R. Minot, J. J. Fulton, H. F. Ulrichs, Florence C. Sargent, J. H. Weare and W. P. Murphy, *Jour. Biol. Chem.*, 74: 69, July, 1927.

[6] M. Gansslen, *Klin. Wchnschr.*, 9: 2099, 1930.

[7] W. B. Castle and F. H. L. Taylor, *Jour. Am. Med. Assoc.*, 96: 1198, 1931.

[8] I. Zadek, *Ztschr. f. Klin. Med.*, 95: 66, 1922.

[9] Wm. B. Castle, W. C. Townsend and C. W. Heath, *Am. Jour. Med. Sci.*, 180: 305, Sept., 1930.

mal gastric secretions which is closely related to the normal mechanism of red blood cell formation. Furthermore, this substance must react with some article of diet (the extrinsic factor which is likewise unidentified) to form a heat-stable material which is stored in the liver, and released as needed to regulate the rate of maturation of red blood cells in the bone marrow. These known facts compel us to conclude that the immediate cause of pernicious anemia is a lack of the intrinsic factor in the gastric secretion, but the factor which is responsible for this still remains obscure. Recent work at the Simpson Memorial Institute[10] indicates that the intrinsic substance is not entirely absent in the gastric juice of all patients with pernicious anemia, but is present in inadequate amounts. This can be demonstrated by collecting considerable quantities of gastric juice from patients with pernicious anemia and after incubating large quantities of it with Hamburg steak, inducing a remission by administering it to patients with the disease.

Pernicious anemia has been called a "macrocytic anemia" because a majority of the red blood cells of the peripheral blood are larger than normal. There are several conditions, however, which will produce a similar type of anemia. This is apparent when the normal mechanism which regulates the rate of red blood cell production is considered. The substance which controls this is the end result of a series of at least four essential steps. If any one of these is lacking, the red blood cells will not maturate properly, and a macrocytic anemia will result. The first essential to the normal development of blood is the ingestion of an adequate amount of material in the diet which has been called the "extrinsic factor." When this is lacking, as it probably is in certain tropical anemias, a macrocytic type of anemia results. Second, this variety of anemia will result when there is an absence of the intrinsic factor in the gastric secretions. As previously stated, this is known to occur in true Addisonian pernicious anemia. Third, although the extrinsic and intrinsic factors may be present and interact properly, the elaborated product may not be absorbed normally and the characteristic anemia will appear. This has been known to occur, for example, in a small group of patients who have had multiple intestinal anastomoses for some surgical reason. As a result there may be only a small portion of the intestines available to absorb this blood-regulating substance and, therefore, the amount which is made available to the body may be inadequate. Finally, a macrocytic anemia may result, following wide-spread liver damage which seriously impairs the storage capacity of this organ. While the material which regulates the maturation of the red blood cells is made in the stomach, it is stored in the liver, where it is released as required to regulate the orderly rate of red blood cell production. As a result of certain diseases, such as cirrhosis of the liver, this latter function may fail and a macrocytic anemia appear.

The work of Castle suggested to Sturgis and Isaacs that possibly gastric tissue itself should be potent in the treatment of pernicious anemia. In 1929[11] they demonstrated that desiccated, defatted hog stomach (Ventriculin) had a similar action to liver and that it was an effective form of therapy in this disease.

This group of experiments which have just been discussed have, therefore, furnished effective methods of controlling a disease which was hitherto invariably fatal, and also have provided additional information of prime importance which may eventually lead to a complete cure by the elimination of the fundamental cause of the condition.

I now wish to turn to an entirely different type of anemia but also a condition about which our knowledge has increased greatly during the past few years. I refer to the iron-deficiency anemias which have in common a depletion of the iron reserves of the body due to various causes.

While no attempt will be made to discuss the metabolism of iron in the body, the following facts should be emphasized. The entire iron content of the body of an average human adult is estimated to average about 3 grams. Eighty per cent. of this is found in the blood serum and in combination with hemoglobin, which contains 0.335 per cent. The average iron intake of an adult in the United States is commonly accepted as about 15 mg. daily and of this amount about one half is eliminated in the feces. Only the slightest trace, if any, is excreted through the kidneys. The body possesses a remarkable mechanism for the conservation of iron, as it is used over and over again. In addition, if the iron intake is reduced sufficiently, the amount eliminated in the stools is decreased and may entirely disappear.

As iron is an essential part of the hemoglobin molecule, it is perfectly clear that this essential material can not be synthesized in the absence of the metal, and an anemia will develop. Let us then consider the conditions which may result in the depletion of the iron stores in the body and a resultant anemia.

An iron deficiency of the body may result when there is an insufficient intake of this metal in the diet. An anemia of this type rarely occurs in adult males, but it is common in infants, women and in growing children. It has been clearly established that adult males will not develop an anemia when the iron intake is as small as 6 mg. daily. In women such fac-

[10] Raphael Isaacs and S. Milton Goldhamer, *Proc. of the Soc. for Exp. Biol. and Med.*, 31: 706, 1934.

[11] Cyrus C. Sturgis and Raphael Isaacs, *Jour.* Am. Med. Assoc., 93: 747, Sept., 1929.

tors as menstruation, pregnancy and lactation cause increased demands for iron and the reserves of this metal will be reduced and an anemia appear, if there is a deficiency of dietary iron. This is also true of children during a period of rapid growth, especially if there is an associated infection.

Another cause for a deficiency of iron in the body is seen in women between the ages of 20 and 40 years who apparently are unable to absorb iron in adequate amounts, despite the fact that a sufficient quantity is present in the diet. Such a condition gives rise to changes in the blood which are designated as idiopathic hypochromic anemia of women, characterized by a red blood cell count which is not strikingly changed but a hemoglobin percentage which may be 50 per cent. of normal or less. While the entire mechanism concerning the absorption of iron is not known, it has definitely been established that hydrochloric acid in the gastric contents favors the absorption of this element, whereas the absence or diminution of this acid impairs it. That the absence of it may play a rôle in the production of this type of anemia is indicated by the fact that all patients with this disease have an achlorhydria.

Probably the most common type of iron-deficiency anemia is observed when there is an excessive loss of this metal from the body, such as occurs in any condition causing chronic hemorrhage. Even a small daily loss of blood, if continued constantly for months, will gradually but severely deplete the iron reserves of the body. If the cause of the hemorrhage is controlled and the patient is partaking of a diet which is not poor in iron, there will be a gradual regeneration of red blood cells and hemoglobin until the normal is reached. Such a condition is a definite therapeutic indication for the administration of iron, as it will greatly facilitate the return of blood to normal. In the anemia which results from uncomplicated acute hemorrhage, however, iron does not have a therapeutic effect, as the normal reserve stores of this substance in the body are usually adequate to accomplish a regeneration of hemoglobin at the maximum rate.

In past years there has been a great deal of discussion concerning the therapeutic value of various forms of iron. At present it appears to be definitely established that the effectiveness of any given form of iron is directly proportionate to its metallic content. I prefer to use, therefore, reduced iron which contains 90 per cent. of the element. Ferric ammonium citrate, which contains approximately 16 per cent. of metallic iron, is also satisfactory. In order to produce satisfactory results, however, the dosage of the preparations must be much larger than is ordinarily advised. For reduced iron the maximum therapeutic dosage is 0.5 grams three times daily and for ferric ammonium citrate it is 2 gm. three times daily. When large doses of iron are administered, a very large percentage of it is excreted in the stools, and the reason why such a large dose is required to produce a therapeutic effect is not known.

The third blood disease for discussion is a condition which has been recognized only in recent years. In 1922 Warner Schultz[12] described what he regarded to be a new clinical entity, characterized by ulcerative lesions of the mucous membranes of the mouth and throat, a marked reduction or complete disappearance of the polymorphonuclear neutrophil cells of the peripheral blood, marked prostration and a rapidly fatal termination. He called this condition agranulocytosis, but later the name agranulocytic angina was given it and more recently other names such as granulopenia, granulocytopenia, malignant neutropenia and others have been applied to it. Historically at least, the terms agranulocytosis and agranulocytic angina have the preference and for that reason will be used in this article.

The mechanism of the production of this disease appears to be as follows. Some unknown agent causes the polymorphonuclear neutrophil cells of the peripheral blood to diminish in number or disappear completely either by depressing the action of the bone marrow where they are formed or by causing an increased destruction of them in the blood stream or possibly by a combination of these two factors. As a result, one of the major defense mechanisms against infection is impaired. This permits the pathogenic organisms which are always present on the mucous membrane surfaces of the body to invade the tissues, producing ulcerative lesions and, in some instances, to develop in the blood stream which almost always results in a fatal termination. While some patients may not have severe symptoms in association with this disease, it must always be regarded as a serious condition because the mortality in the untreated cases is almost 75 per cent. When it is recognized in its early stages and pentnucleotide therapy and blood transfusions are given, and these are the most promising forms of treatment, the mortality has been reduced to about 25 per cent.

For over a decade after Schultz had directed the attention of the medical profession to this disease, the cause of the reduction or disappearance of the polymorphonuclear neutrophil cells from the blood was undetermined nor was it possible to account for the characteristic tendency of these patients to suffer from relapses.

In October, 1933, Madison and Squier,[13] however,

[12] W. Schultz, *Deutsche med. Wchnschr.*, 48: 1495, 1922.

[13] F. W. Madison and T. L. Squier, "Primary Granulocytopenia after Administration of Drugs Containing a

reported before the Central Society of Clinical Investigation that the syndrome of agranulocytosis might be precipitated by the use of drugs composed of a combination of a barbiturate with amidopyrine. This type of medication is often prescribed by physicians and is used more and more by the non-medical public on their own initiative. These investigators observed that 14 of their patients with the disease had taken amidopyrine alone or in combination with other drugs, such as a barbiturate, immediately before the onset of the disease. This report immediately aroused my interest in this possible etiological relationship and as a result a study was made of our cases of the disease at the University of Michigan.[14] Ten patients have been observed in this hospital and it was definitely established that all of them had taken drugs or combinations of drugs containing amidopyrine in a short time before the earliest symptoms appeared. Although these observations and those contained in a number of other subsequent reports indicate clearly that patients with this disease frequently take amidopyrine before the onset of the condition, it is only indirect evidence that there is a causal relationship between this drug and agranulocytosis. More convincing data have been obtained by administering small doses of amidopyrine orally to patients who had recovered from the disease and determining the white blood cell count every half hour for four hours. In each one there was a striking decrease in the number of polymorphonuclear neutrophil cells of the peripheral blood which reached a maximum in 1½ hours and then returned to normal at the end of 4 hours. A repetition of the test in two of the patients produced exactly the same effect. These tests were controlled by observing the white blood cell count every half hour in these same patients, during which time no drug was given. Furthermore, the administration of this drug to two normal persons did not produce significant changes in the leucocyte count during a four-hour interval.

It is my opinion that amidopyrine is the drug which precipitates the disease in certain persons who are susceptible to it. As it is a widely used therapeutic agent and agranulocytosis does not have a high incidence, it must be concluded that the percentage of persons who are sensitive to it is not great.

WHAT IS A PROOF?[1]

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OUR first notion of proof may have been different from person to person, but I dare say that for none of us was it the logical process and the Q.E.D. of geometry; for all of us it was probably something quite authoritarian which first brought conviction—a reiterated statement, a punishment, an emphasis, possibly just the stamp of a foot, maybe an example, a bit of cajolerie, a reward or merely an acquired habit. This type of proof is not to be ignored, it is widely and effectively used to demonstrate the excellence of a cigaret, the indispensability of a governmental measure or the soundness of a social theory. Thus, if we were to give to the term proof a definition which had any wide validity in human affairs we should have to use some such phraseology as "a process by which A induces in B a sense of the justification for a conviction."

We may remark that A and B may be the same individual. We should note that the proof is relative to B in whom the sense of justification for the conviction is induced by the process. When a class in plane geometry first meets the Pythagorean Theorem (Euclid I, 47) with its complicated auxiliary construction lines and lengthy reasoning, the proof given will not be such to most of the youngsters because it does not carry to them a sense of justification, it is blind. Or, again, when a class in differential calculus reaches the subject of maxima and minima, the teacher with a few necromantic passes, verbal and graphical, may prove in a manner which carries both a conviction and the sense of its justification, the rule that to find maxima or minima of a function the derivate is set equal to zero; yet he knows that the theorem as stated is neither proved nor true. What may be a perfectly good proof to B may be none to A, who gives it, and a perfectly good proof to A may be none to B, who receives it.

That which has been illustrated relative to proofs of familiar propositions, holds equally of facts, as may be seen by reading "Fact: The Romance of the Mind," the latest book of Henry Osborn Taylor, our very illustrious historian of thought, wherein you can learn that what at some time has been considered indubitable fact might not be so considered now—

Benzene Chain,'' presented at annual meeting of Central Society for Clinical Research, Chicago, Oct. 27, 1933.

[14] Cyrus C. Sturgis and Raphael Isaacs, ''Observations concerning the Etiology of Agranulocytosis'' (*Trans. of the Assoc. of Amer. Physicians*, 49: 328, 1934).

[1] Read at the Pittsburgh meeting of the American Association for the Advancement of Science, before a joint session of Sections K and A with the American Mathematical Society and the Mathematical Association of America and the Econometric Society.