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SOME ASPECTS OF THE PATHOLOGY OF ANAEMIA

I. THEORY OF MATURATION ARREST*

BY

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I have chosen as my subject "Some aspects of the pathology of anaemia" because the phrase recalls the title of my Goulstonian Lectures on the pathology and treatment of anaemia in 1932 and I thought it would be of interest in the present lectures to discuss how our ideas have changed since that time (Witts, 1932). I shall, however, confine myself to the anaemias from deficiency of iron, vitamin B_{12}, and folic acid—in other words, the hypochromic and megaloblastic anaemias, and I shall omit the haemolytic anaemias, about which much of what I wrote in 1932 was erroneous. Their elucidation is one of the most brilliant chapters of haematology; indeed, the subject has expanded from a chapter to the two volumes of Professor J. V. Dacie's (1960) invaluable book, *The Haemolytic Anaemias*, so that any remarks of mine would be completely nugatory.

Hypochromic anaemia is a descriptive term, as Asher (1959) has emphasized, and it is not synonymous with iron-deficiency anaemia. There are other varieties of hypochromic anaemia, such as Mediterranean anaemia, pyridoxine deficiency, and the sidero-achrestic or refractory normoblastic anaemias, in which there is inability to synthesize haemoglobin in spite of a plentiful supply of iron (Dacie, Smith, White, and Mollin, 1959; Heilmeyer, 1959). Here, however, the only hypochromic anaemia I shall be concerned with is that from deficiency of iron. Of course, blood cells are not just made of iron, vitamin B_{12}, and folic acid. A large number of other factors are necessary for their manufacture, but these are not so closely concerned with blood formation, and even vitamin C, deficiency of which can sometimes produce striking alterations in the blood, can be regarded as acting through the conversion of folic to folinic acid. The generic name for the anaemias from deficiency of haematinic factors used to be the "dyshaemopoietic anaemias," but it has never become popular, perhaps because it is not euphonious rather than because of any disagreement with the theory from which the word was derived. Nevertheless, the first aspect of the pathology of these anaemias I should like to discuss is what we meant in the past and what we mean to-day by dyshaemopoiesis.

The Theory of Maturation Arrest

Haematology in the years immediately after the first world war was characterized by the development of the concept of the erythron and the increasing use of quantitative methods such as the determination of the blood volume and the total red-cell mass, and the measurement of the various red-cell constants such as the mean corpuscular haemoglobin concentration. Nevertheless, in 1932 it was still possible for me to write, "Haematology will not be an exact science until we can measure the red cells, which enter or leave the circulation in the 24 hours, with the same accuracy as we can measure the protein metabolism or the work of the heart." We have made substantial progress in this direction but can as yet hardly claim that ours is an exact science.

In a reaction against the tendency to attribute all unexplained anaemias to toxins and haemolysins it was postulated that the anaemia and the hyperplasia of the marrow in hypochromic anaemia and pernicious anaemia were due to a futile overgrowth and dysplasia of the marrow which was accompanied by a diminished output of cells. The analogy was with the hyperplasia of the thyroid gland in simple goitre from iodine deficiency. A similar hypothesis of maturation arrest was applied to agranulocytosis and thrombocytopenia. The idea that the haemopoietic cells could be frozen into immobility, like the attendants of the Sleeping Beauty, and could be magically restored to activity by the appropriate haemopoietic principle was a picturesque one. Other similes were the motor-car factory, with unfinished cars piling up for want of an essential component, and the training area from which no trained troops emerged because the soldiers had been equipped only with left-sided boots.

Our Treasurer has justifiably poked fun at these similes and has shown that the theory of maturation arrest left many of the features of pernicious anaemia unexplained, particularly the slight icterus and the great increase in excretion of urobilinogen (Bomford, 1946). Moreover, it has now been pretty convincingly demonstrated that when thrombocytopenia and agranulocytosis are not due to aplasia of the marrow, they are due to increased destruction of platelets or white cells.

The antiglobulin reaction or Coombs test, which was introduced by Coombs, Mourant, and Race in 1945, made it possible to demonstrate abnormal antibodies on the surface of the patient's erythrocytes or in his serum in the acquired haemolytic anaemias. This was soon followed by evidence that acquired haemolytic anaemia, idiopathic thrombocytopenic purpura, and agranulocytosis might be regarded as sections of a continuous

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spectrum of disease (Evans, Takahashi, Duane, Payne, and Liu, 1951). They may occur simultaneously or successively in the same patient. In all three disorders the marrow is hyperplastic, with an excess of immature precursors of the red cells, white cells, or platelets respectively, and there is a great diminution of mature forms. Ackroyd (1949) demonstrated a platelet antibody reaction in sedormid purpura, and Harrington and co-workers (1951) showed that plasma from patients with idiopathic thrombocytopenic purpura reduced the platelet count in normal subjects. It is now accepted that there is greatly increased destruction of platelets in idiopathic thrombocytopenic purpura (Ackroyd, 1955). Similarly Moeschlin (1958) has described anti-leucocyte factors in agranulocytosis due to drugs and has shown that the injection of anti-leucocyte sera in animals will produce a marrow picture similar to that of amidopyrine agranulocytosis in man. This buttress of the theory of maturation arrest has therefore collapsed.

Measurement of Red-cell Survival

The increase in the number of blood transfusions and in the amount of blood transfused, which had started before the beginning of the second world war, sent us back to a reappraisal of Winifred Ashby's (1919) method of measuring the life-span of the red cells by transfusing blood of a different but compatible group, and it was soon realized how great was its value. I had been interested in Schiødt's (1938) observations on the regeneration of the blood after haemorrhage from peptic ulcer. Schiødt was the first person to treat these problems mathematically, and though his figure of 33 days for the life of the red cells was wrong, his hypothesis that the normal destruction of the red cells could be explained either by the theory of longevity, according to which the red blood cells have a definite expectation of life and die when they reach a certain age, or by the theory of destruction, according to which a definite fraction of the red-cell population is arbitrarily destroyed every day, or by a combination of both theories, has dominated our ideas to the present day.

We were fortunate to interest a mathematical friend, Mr. E. O. Powell, in the curves we had obtained with the Ashby method in Oxford, and he carried out the first, I think, of the mathematical studies of red-cell survival which have subsequently made the subject of erythrokinetics a playground for medical mathematicians and have tended to frighten away those of us who have no head for figures (Brown, Hayward, Powell, and Witts, 1944; Witts, 1950). In the Ashby technique the blood group is used as a marker. The introduction of less tenacious markers such as radiochromium has made the mathematics even more complicated. Nevertheless, the ability to label the patient's own cells with radiochromium, and to track them during life by scintillation counting, in their peregrinations through the liver and spleen as well as in the blood-stream, has greatly increased the amount of information which can be obtained from studies of red-cell survival (Aufderheide, 1960; Belcher and Hughes Jones, 1960). This information can usually be translated into simple words and curves, and the subject need not really be terrifying. It has, in fact, been the keystone in our understanding of the haemolytic anaemias and the great new structure of knowledge to which I have just referred. We can now determine with considerable accuracy whether abnormal haemolysis is occurring, its extent, and whether it is due to faults in the red cell or to abnormalities in the plasma or the viscera through which it circulates.

The normal human red cell has a life-span of 110 to 120 days. Even in health not all the cells attain the allotted span of days, for the red cell, like man himself, may suffer accident. In disease the rate of destruction of the red cells may be increased by premature senescence or by an increase of the accidents which we call haemolysis and which may affect the red cells independently of their age. The red cells of patients with pernicious anaemia have a much shortened life-span, only about half the normal. It is also probable that normal blood cells are destroyed abnormally rapidly when they are transfused into patients with pernicious anaemia (Berlin, Waldmann, and Weissman, 1959). This means that pernicious anaemia could be classified as a haemolytic anaemia. However, the rate of haemolysis is not so rapid as in acholuric jaundice or acquired haemolytic anaemia of comparable severity, so that haemolysis cannot be the whole story.

Little work has been done on the longevity of the red cells in the anaemia of iron deficiency. We might expect from their departure from the ideal form and their fragile appearance that their expectation of life would be diminished. Mollison (1956) thinks that it is normal, and that is our impression from work in Oxford. One of the difficulties in studying the life of the red cells in hypochromic anaemia is to ensure that the anaemia is not due to persistent bleeding, which will of itself reduce the life-span of the red cell. Taking this precaution, Rasch and co-workers (1958) nevertheless came to the surprising conclusion that the life-span of the red cells in the iron-deficiency anaemia of infants was reduced to almost half the normal value. Verloop, van der Wolk, and Heier (1960) reported a smaller but still significant reduction in the life-span of the red cells of women with hypochromic anaemia and iron deficiency. Clearly the last word has not yet been said on this subject.

Measurement of Red-cell Production

In a steady state of haemopoiesis, whether in health or disease, blood production balances blood destruction, and the red-cell-survival curve is therefore an indirect measure of the rate of blood formation. The direct determination of the rate of red-cell formation has proved more difficult. It has been attempted by studying the incorporation of isotopes in the newly formed red cells. The results which seem most reliable have been obtained by administering glycine labelled with ^{15}N or ^{14}C, and studying the concentration of the isotope in the haem pigment in the red cells and the biliary pigment in the stools (Shemin and Rittenberg, 1946). When labelled glycine is administered some of it is incorporated in the haemoglobin of the red cells which are formed contemporaneously. The isotope is fixed in these cells and does not participate in the dynamic equilibrium of the body. When this particular cohort of red cells is broken down the isotope remains in the stercobilin which is derived from the haemoglobin and is excreted with it from the body.

Unfortunately, the apparatus for estimating the concentration of the isotope is expensive and complicated and the extraction procedures and measurements demand much skill. The total number of cases studied

by this method is small, too small perhaps for the important theoretical structure which has been built on the results. The curve of appearance and disappearance of the labelled haem pigment in the red cells is just what we should expect from our previous studies, and analysis of the curves gives the same life-span of 110 to 120 days. The curve of pigment excretion, however, is surprising, for in addition to the expected peak of excretion of labelled stercobilin soon after 100 days, when the cohort of labelled red cells has aged and died, there is an early peak of excretion of labelled stercobilin within a few days of the administration of the labelled glycine. This peak is considerably elevated in pernicious anaemia and indeed in a number of other anaemias such as congenital porphyria and Mediterranean anaemia (London and West, 1950; Grinstein, Bannerman, Vavra, and Moore, 1960). Unfortunately I have not been able to find a report of a patient with chronic iron-deficiency anaemia studied by this technique.

Concept of Ineffective Erythropoiesis

These studies with labelled glycine and particularly the demonstration of the early peak of pigment excretion have led to the conclusion that 10% of the stercobilin is derived from isotopically labelled precursors which are not the haemoglobin of circulating mature red cells. Going beyond this conclusion it has been thought that the red-cell population may normally be composed of two different fractions which undergo destruction at greatly different rates. Bile pigment may be formed not only from aged worn-out erythrocytes but also from the haemoglobin of erythrocytes which are destroyed in the bone-marrow before reaching the peripheral blood, or shortly after entering the circulation. This is the concept of "ineffective erythropoiesis" which has become a major element in our thinking about the maintenance of the red-cell level in health and the failure to maintain it in pernicious anaemia and iron deficiency. I must stress that ineffective erythropoiesis is still only a hypothesis, and other explanations have been offered for the shape of the curve of pigment excretion.

Although the destruction of a proportion of the erythrocytes round about the time of their birth at first sight seems an odd phenomenon, this may be because of our habit of anthropomorphizing the cells and regarding them as little entities which are entitled to live out their span of days in the *milieu interne*. The body, however, uses cells as it thinks fit, if I too can speak in anthropomorphic terms, and makes them for the briefest of life-spans in other situations such as the surface of the stomach and intestine. It is probable, in fact, as I shall mention later, that ineffectual erythropoiesis is one of the stabilizing mechanisms of the erythron which enables it to respond freely to the varying demand for circulating erythrocytes.

Ferrokinetics

The other method which has been used to measure the rate of blood formation is the administration of a tracer dose of radioiron (Huff, Hennessy, Austin, Garcia, Roberts, and Lawrence, 1950). The dose is injected intravenously and the rate of clearance of the radioiron from the plasma is measured. Most of the tracer dose subsequently reappears as haemoglobin in the red cells, and it is usual to determine the percentage utilized for red-cell formation and the time between the injection and the maximum daily rise of radioactivity in the red cells. From these data the fraction of the red cells renewed per day can be calculated, and the values obtained for normal individuals are in good agreement with those of the other methods already discussed. In contrast to the use of labelled glycine, the use of radioiron is technically easy and a large number of individuals have been studied. The difficulty lies in the interpretation of the results, for iron is an element which is not limited to a single cycle of blood formation and its movements in the body are devious and involved. A number of supplementary data must be obtained to enable one to interpret a particular situation.

It is believed that the plasma iron turnover is the best available means of assessing the amount of erythropoietic marrow, and as there is, as we shall see later, little or no concrete evidence of maturation arrest, this means that the iron turnover is likewise a measure of total marrow activity. This is not the same thing, however, as saying that the iron turnover is a direct measure of effective blood formation. Indeed, the results obtained in disease are incompatible with such a view. In pernicious anaemia Clement Finch and his colleagues found that there was a dissociation between the erythropoietic indices derived from the reticulocytes and the red-cell radioiron utilization, which were subnormal, and the indices derived from the erythroid–myeloid ratio, the plasma iron turnover, and the stercobilin, which were three to six times normal (Giblett, Coleman, Pirzio-Biroli, Donohue, Motulsky, and Finch, 1956). In idiopathic hypochromic anaemia we found in our laboratory that, although the concentration of iron in the plasma was low, the plasma iron turnover was normal or increased (Bothwell, Callender, Mallett, and Witts, 1956). Similarly, in Finch's department both the plasma iron turnover and the reticulocyte production were found to be increased in iron-deficiency anaemia (Bothwell, Hurtado, Donohue and Finch, 1957).

Nature of the Dyshaemopoiesis in Pernicious Anaemia and Iron Deficiency

The explanation of these findings is still rather speculative. In pernicious anaemia it would appear from the radioiron studies that the total marrow activity is increased to about three times normal but that only a third of the cells produced enter the circulation. In other words, the short-lived component of the red-cell population which is destroyed almost immediately after manufacture is greatly increased. With therapy the rate of erythropoiesis is not at first greatly altered; what happens is a conversion from largely ineffective to effective erythropoiesis (Finch, Coleman, Motulsky, Donohue, and Reiff, 1956). In folic acid deficiency the pattern is similar but less striking (Sheehy, Rubini, Baco-Dapena, and Perez-Santiago, 1960). All this is in keeping with the clinical picture in the megaloblastic anaemias, the jaundice in relapse, and the correlation of the severity of the anaemia, the degree of hyperplasia of the marrow, and the height of the reticulocyte crisis on remission. It is also in keeping with the observations with labelled glycine.

The results in idiopathic hypochromic anaemia, which suggest erythropoietic activity within the normal range in spite of the anaemia, are hard to understand. They have led to doubt whether tracer studies with radioiron

are appropriate for the measurement of the rate of blood formation in anaemia due to iron deficiency. Pollycove (1959), who uses elaborate methods for studying the clearance of radioiron and its utilization in red-cell formation, finds that the mean red-cell life-span, as calculated from the daily haemoglobin synthesis, is shortened to 44 days in idiopathic hypochromic anaemia, but he suggests that this is largely due to the breakdown of maturing red cells within the marrow and that the life-span of the circulating erythrocytes is only slightly reduced. In other words, the anomalous findings in idiopathic hypochromic anaemia also are due to ineffective erythropoiesis. However, of this we have no collateral evidence from other techniques as we do for pernicious anaemia and folic-acid deficiency, nor is Pollycove's interpretation supported by the observation that the bone-marrow responds to treatment with iron with intensified hyperplasia (Beutler, Drennan, and Block, 1954).

The New Model of the Erythron

In view of the difficulties with radioiron it may seem surprising that more work has not been done on the measurement of blood formation in health and disease by means of labelled glycine. However, there are fashions in scientific research as in the arts, and workers have tended to turn from these large-scale studies to the analysis of the successive enzymatic reactions whereby the nuclei of the blood cells and other cells are built up. But, quite apart from this, it has been learned that progress in biological research is likely to be more rapid if we can devise a model of the process under study, either a mathematical model such as Norman Bailey's (1957) model for the study of epidemics or a physical model such as the electronic computers with which neurological research workers compare the brain. The devising of models based on the whole complicated structure of the blood, the blood-forming organs, and the body stores has proved unsatisfactory. For this reason the younger generation of haematologists have tended to confine their attention to the erythron itself and have been caught up in the study of what is called the kinetics of cellular proliferation (Stohlman, 1959).

It is possible to label the erythroid cells with radioiron and the myeloid cells with radiosulphur, and all the haemopoietic cells can be labelled with radiophosphorus, tritiated (^{3}H) thymidine, ^{14}C thymidine, and other potential constituents of the cell, so that autoradiographs can be prepared or the different components of the cells can be isolated and their radioactivity determined. By such methods it is hoped to discover the proportion of cells which differentiate and mature, the various levels of differentiation, and the duration of the mitotic and intermitotic cycles. In this way a model of the erythron can be devised (Lajtha and Oliver, 1960), and this can be used not only to help us to measure the rate of blood formation in health and disease but also to discover the nature of the disturbance in the cell generations in conditions such as pernicious anaemia.

The main regulator of erythropoiesis is the rate of differentiation of erythroblasts from the stem cells under the influence of the hormone "erythropoietin," which is one of the main foci of haematological research at the present time (Gordon, 1959). Once the erythroblasts have begun to differentiate it seems unlikely that the process can be arrested or that the rate of proliferation and maturation of the dividing erythroblasts can be greatly retarded. The weight of opinion is against the possibility of maturation arrest. However, the question is not yet finally resolved, and in Mediterranean anaemia it has been claimed that the rate of division of the later erythroblasts and the ripening of the reticulocytes are slower than normal (Salera, Tamburino, and Magnanelli, 1957; Baldini and Pannacciulli, 1960).

A secondary regulator of erythropoiesis is the intricate balance between cellular differentiation and ability to divide. Slight disturbance of this balance may enable the cells to mature more rapidly, and this mechanism may play a part in the prompt response to loss or destruction of blood. Any serious unbalance between cellular differentiation and ability to divide, which probably means between cytoplasmic and nuclear functions, may cause cell death at an early stage, and this probably corresponds to the process already described as ineffective erythropoiesis. Workers in this field likewise believe that some ineffective erythropoiesis—not more than 10%—occurs in health but that the death rate or "abortion rate" of the red-cell precursors may be greatly increased in diseases such as pernicious anaemia.

If we go back to our analogy of the car factory we can imagine that when there is a deficiency of vitamin B_{12} or folic acid large numbers of extremely defective cars are half made and promptly scrapped, and that the cars which are actually delivered are of poor quality and run for only 50,000 miles instead of 100,000 miles. This gives us quite a pretty explanation of the jaundice—from the scrapping and the premature wear and tear of the vehicles or red cells—and of the reticulocyte crisis; when the shortage of raw materials allows the scrapping process to be ended, the factory has so many completed vehicles on its hands that it turns them out before the paint is dry. In iron deficiency it must be assumed that the disturbance of red-cell formation is less profound and there is not enough neonatal or prenatal destruction of red cells to lead to jaundice. In addition, the survival of the red cells which enter the circulation is not necessarily curtailed. Nevertheless, in small animals like the rat, iron deficiency produces considerable hyperplasia of the marrow and reticulocytosis, and in the profound iron-deficiency anaemias which occur in pregnant women in Bengal the picture can be quite like pernicious anaemia.

These models and analogies must not be taken too seriously. The kinetics of cellular proliferation is a young science and there is still much controversy about basic facts such as the life-span of the granulocytes and the lymphocytes. We cannot explain the occurrence of normoblastic and megaloblastic hyperplasia of the bone-marrow from deficiency of vitamin B_{12}, folic acid, and iron as satisfactorily as we can explain the various stages and ways in which the synthesis of thyroxine may break down. Nevertheless, it does seem that hyperplasia of the bone-marrow always implies increased activity, even though some or perhaps much of the activity may be fruitless, and the hypothesis of maturation arrest, which was so useful for a time while we were separating the dyshaemopoietic from the haemolytic and the aplastic anaemias, must now be abandoned or at any rate have its importance much abated.

[The second lecture, with a list of references, will be published next week.]