

MEDICAL RESEARCH WITH RADIOISOTOPES IN GREECE

By E. H. Belcher

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An important program of research into the nature and causes of congenital haemolytic anaemias, notably the disease known as Mediterranean anaemia or thalassaemia, which is a serious medical problem in the Mediterranean countries, is at present being carried out in the Department of Clinical Therapeutics of the University of Athens under a research contract awarded by the International Atomic Energy Agency.* This program is concerned with diseases in which there is an inherited defect or abnormality in the production of haemoglobin, the iron-containing pigment of the red blood cells which is responsible for the carriage of oxygen in the blood.

In normal persons, two forms of haemoglobin with different physical and chemical properties are found. These are adult haemoglobin, often called haemoglobin A, and foetal haemoglobin, haemoglobin F. The oxygen-carrying properties of haemoglobin F are particularly suited to the conditions of relative lack of oxygen in which the human foetus must survive before birth. In the red cells of the unborn child the haemoglobin is largely of this type, but haemoglobin F normally disappears shortly after birth to be almost completely replaced by haemoglobin A.

Inherited Abnormalities

Several inherited abnormalities of haemoglobin production have been recognized. Important among these is Mediterranean anaemia or thalassaemia in which haemoglobin A production appears to be defective; as a result, haemoglobin F persists into adult life. The red blood cells of persons suffering from this disease are small, thin and deficient in pigment. Another important hereditary condition is sickle cell disease, in which an abnormal haemoglobin, haemoglobin S, is produced instead of haemoglobin A. If the red cells of persons with this disease are exposed to lack of oxygen, they undergo a bizarre transformation, first changing in shape from circular to crescentic and finally breaking down completely. These changes, which comprise the "sickling" phenomenon from which the disease takes its name, can occur in the circulation, and the resultant rapid destruction of red cells causes profound anaemia. In yet other hereditary conditions, different abnormal haemoglobins, designated as haemoglobin C, D, E, F, G, H etc., replace haemoglobin A. The exact differences

between these forms of haemoglobin have yet to be established, but their presence in samples of blood may be demonstrated by various tests.

The inheritance of such abnormalities follows simple Mendelian laws. When a person inherits one of these conditions from one of his parents only, he is said to carry the trait of the disease and usually suffers only slight or negligible disability. However, when two such trait carriers marry, an average of one in four of their children inherits the condition from both parents and develops the major form of the disease. This always entails severe anaemia and serious disability, which may be incompatible with survival to adult life.

Geographical Distribution

The geographical distribution of these diseases has led to much controversy about their past spread by migratory movements. Thus, thalassaemia is widespread in all Mediterranean countries. However, a second focus for this condition is found in South-east Asia, and it is interesting to speculate whether the latter is due to past migrations of Mediterranean peoples or represents the original focus of origin. A third possibility is that the disease may have arisen independently in the two regions. Sickle cell disease is found throughout tropical Africa, but also to a lesser extent in Mediterranean countries and in Asia. Haemoglobin E disease has been observed predominantly in South-east Asia. The other abnormal haemoglobins have less clearly defined distributions. A further subject for speculation is the manner in which these abnormal genes have persisted over the years in the face of natural selection. It is difficult to explain how hereditary diseases with high mortality in infancy and childhood can continue to be widespread unless they also carry some positive advantage over the normal state, and it has often been suggested that the continued existence of the thalassaemia trait or sickle cell trait may be due to an associated resistance to malaria or other endemic disease.

Whatever be the reason for their persistence, the congenital haemolytic anaemias present a serious medical problem in many countries. For example, the results of a recent survey in Greece show that more than 7 percent of the population of this country carry the thalassaemia trait, whilst the sickle cell trait and other abnormalities are also quite common. Blood transfusions may save the lives of persons suffering from the major forms of these diseases and

* Similar investigations are also being conducted under Agency research contracts in the Republic Hospital, Baghdad, and at the University of Medical Sciences, Bangkok.

removal of the spleen may be of benefit in certain cases, but no form of the treatment has yet been devised which will correct the basic defects or abnormalities in haemoglobin production. Indeed, these defects are still imperfectly understood and further research investigations into their nature and effects are urgently needed. In such investigations, radioactive tracer techniques have been found especially valuable.

Techniques of Investigation

Two techniques have been widely used in the studies at the University of Athens. In the first of these, a radioisotope of iron, iron-59, is used to follow iron metabolism and haemoglobin production. Iron metabolism in the body is concerned largely with the synthesis and breakdown of haemoglobin, which consists of a protein, globin, linked to an iron-containing substance, haeme. If iron-59 is injected intravenously into a human subject, the isotope normally disappears from the blood within a few hours, being taken up mainly by the maturing red cells in the bone marrow and used by them for haemoglobin production. Within a few days, the mature red cells are released to the circulation labelled with iron-59. The red cells normally survive in the blood for about four months; at the end of their life span, they are removed by the spleen, the liver and other organs, most of their iron-59 being reutilized for the production of fresh haemoglobin. The disappearance of iron-59 from the blood and its subsequent reappearance in the form of labelled red cells may be followed by measurements on blood samples withdrawn from the subject at different times. At the same time, measurements with radiation counters placed at the surface of the body over different organs show the changing distribution of radioactivity in the body, as iron-59 is first taken up in the bone marrow and later appears in the circulating red cells. Such measurements readily reveal any abnormality in haemoglobin synthesis and red cell production.



Measurements of the distribution of radioactive iron in the body being made in the course of investigation at the Alexandra Hospital, Athens, on a patient suffering from thalassaemia

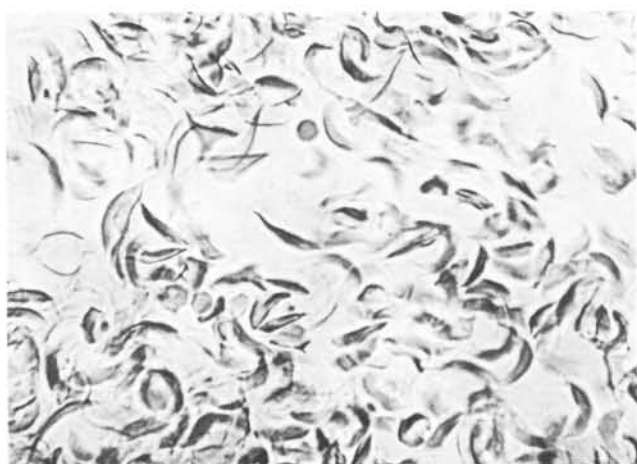
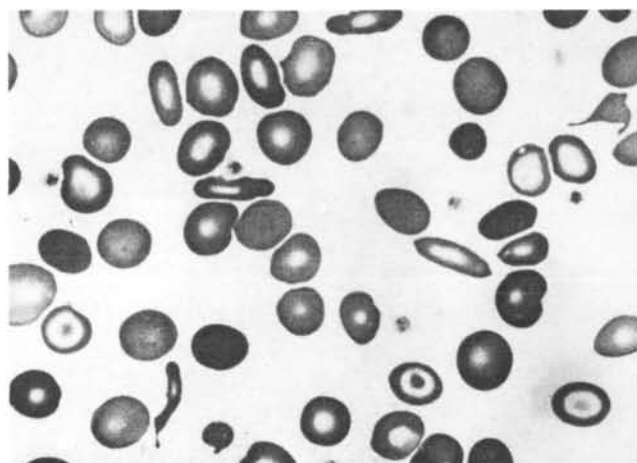
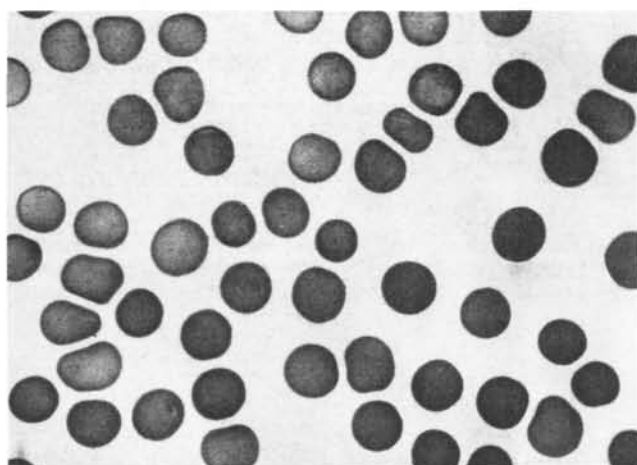
The second technique makes use of a radioisotope of chromium, chromium-51, to study the fate of the red cells in the blood. Red cells may easily be labelled with chromium-51 by adding the isotope under appropriate conditions to a sample of blood. If red cells are labelled in this way and then re-injected into the donor's circulation, the survival of the labelled cells may be followed by measuring the fall in the chromium-51 content of successive blood samples. Furthermore, when the cells are ultimately destroyed, their chromium-51 accumulates in the spleen, the liver and other organs concerned in their destruction. Measurements with radiation counters placed at the surface of the body over these organs indicate their relative importance as sites of red cell destruction.

Results Obtained

By performing simultaneous studies with iron-59 and chromium-51, a detailed picture of haemoglobin synthesis and red cell production and destruction can be built up. Such investigations have been invaluable in establishing the characteristic patterns of different congenital haemolytic anaemias. For example, studies with iron-59 on patients with thalassaemia suggest that in this disease a very high proportion of the developing red cells in the bone marrow fail to reach maturity. These effete cells may be destroyed *in situ* in the marrow, or may reach the circulation in a form so abnormal that they are almost immediately removed by the spleen or other organs. This ineffective production of red cells is very characteristic of thalassaemia and the bone marrow in this disease may be likened to an assembly line working at a very high rejection rate. A few nearly normal cells are, however, produced; studies with chromium-51 show that these cells may survive nearly normally in the circulation and that their ultimate fate resembles that of the red cells of healthy persons. The severity of the disease in fact appears unrelated to the survival of these cells, but depends on the proportion of effete cells produced by the bone marrow. These findings suggest that the treatment of thalassaemia should be aimed particularly at improving the degree of effectiveness of red cell production in the marrow rather than at improving the survival of the red cells in the circulation.

This situation may be contrasted with that found in sickle cell disease. Studies with iron-59 on patients with this disease show that red cell production is highly effective and that very large numbers of red cells are released from the bone marrow to the circulation. Studies with chromium-51 reveal, however, that the survival of these cells is very short, probably as a result of sickling taking place in the circulation. Indeed, in severe cases, the life span of the circulating red cells may be only a few days. The role of the spleen in accelerating red cell destruction in sickle cell disease is noteworthy. Body surface counting studies with chromium-51 show that this organ may be very active in causing sickling and premature destruction of cells, especially in young children.

From these results, it would seem that the treatment of sickle cell disease should be aimed at



The picture on top is of normal red cells in the blood. The one in the middle shows the appearance of red cells in thalassaemia, while the picture at bottom is of red cells in sickle cell disease

(By courtesy of Dr. J.C. White)

preventing the changes leading to the sickling phenomenon and to the destruction of the red cells in the circulation, rather than at increasing the already highly effective production of red cells in the bone marrow.

Further studies have established the characteristic patterns of iron metabolism, red cell production and red cell destruction in combinations of thalassaemia and sickle cell disease, in haemoglobin H disease and other abnormal conditions. A more detailed investigation of abnormal haemoglobin synthesis in these diseases is now being carried out, in order to pinpoint more clearly the basic defects or abnormalities.

The signs and symptoms which confront the physician are frequently confusing, and radioisotope techniques can often help to establish an accurate diagnosis in the individual case. But perhaps their greatest value is that they provide a means of obtaining a deeper knowledge of the fundamental causes of disease. The methods of investigation developed at the University of Athens and the results of the researches carried out there contribute to medical science by providing general methods for the study of blood diseases by radioisotope techniques. Particularly, by promoting a more complete understanding of the congenital haemolytic anaemias, they may perhaps point the way to effective methods of treatment of these diseases.