virus diseases, including many that are arthropod-borne, in which high concentrations of virus are attained in the blood-stream. It seems possible that some epidemics of jaundice not due to yellow fever—for example, that described by Hahn and Bugher (1953)—may have occurred as a result of haemolysis in enzyme-deficient subjects. The observations that some patients had dark urine, and that males were more severely affected than females, are consistent with this interpretation. A further search for haemolysis associated with virus infections should be made.

This discussion has been concerned with some of the selective agents likely to be operating under natural conditions. They have left in African and other populations a genetic legacy that is also a medical hazard, since G6PD-deficient subjects are liable to haemolysis when exposed to a wide range of drugs in therapeutic concentrations. These include the antimalarial 8-aminoquinolines and sulphonamides (Beutler, 1959), and also nitrofurazone, which is used in trypanosomiasis (Robertson, 1960). Indeed, it appears likely that many of the alleged cases of blackwater fever reported in immune Africans are due to severe haemolytic episodes in G6PD-deficient subjects; several such cases in Nigeria have been found by Gilles (private communication) to have enzyme deficiency.

Summary

P. falciparum malaria parasite rates and densities were found to be significantly lower in young African children with erythrocyte glucose-6-phosphate dehydrogenase deficiency than in children with normal enzymes. Reasons are given in the discussion why this might be so. This fact helps to explain why the enzyme-deficiency trait remains common in malarious areas even though it predisposes carriers to haemolysis under certain conditions.

The enzyme tests were carried out in laboratory space provided through the courtesy of the late Dr. D. B. Wilson, at Amani, Tanganyika. The visit of one of us (A. C. A.) to East Africa was made possible by a grant from the Colonial Development and Welfare Fund, recommended by the Colonial Medical Research Committee (now the Tropical Medicine Research Board).

REFERENCES

- Allison, A. C. (1954). *Brit. med. J.*, **1**, 290.
 — (1957). *Exp. Parasit.*, **6**, 418.
 — (1960). *Nature (Lond.)*, **186**, 531.
 Beutler, E. (1959). *Blood*, **14**, 103.
 Carson, P. E., Flanagan, C. L., Ickes, C. E., and Alving, A. S. (1956). *Science*, **124**, 484.
 Childs, B., and Zinkham, W. H. (1959). *Ciba Foundation Symposium on Biochemistry of Human Genetics*, edited by G. E. W. Wolstenholme and C. M. O'Connor, p. 76. Churchill, London.
 Davidson, G., and Draper, C. C. (1953). *Trans. roy. Soc. trop. Med. Hyg.*, **47**, 522.
 Field, J. W. (1949). *Ibid.*, **43**, 33.
 Fulton, J. D., and Grant, P. T. (1956). *Biochem. J.*, **63**, 274.
 Geiman, P. M. (1951). *Parasitic Infections in Man*, edited by H. Most. Columbia University Press, New York.
 Hahn, R. C., and Bugher, J. C. (1953). *Trans. roy. Soc. trop. Med. Hyg.*, **47**, 77.
 McGhee, R. B., and Trager, W. (1950). *J. Parasitol.*, **36**, 123.
 Mandel, S. P. H. (1959). *Nature (Lond.)*, **183**, 1347.
 Marks, P. A. (1960). *Proceedings of 1st Macy Conference on Genetics*, p. 199.
 — Johnson, A. B., and Hirschberg, E. (1958). *Proc. nat. Acad. Sci. (Wash.)*, **44**, 529.
 Motulsky, A. G. (1960). *Proceedings of Conference on Genetic Polymorphisms and Geographic Variations in Disease*, edited by B. S. Blumberg. Public Health Service, Washington.
 Raper, A. B. (1956). *Brit. med. J.*, **1**, 965.
 Rimon, A., Askenazi, I., Ramot, B., and Sheba, C. (1960). *Biochem. biophys. Res. Commun.*, **2**, 138.
 Robertson, D. H. H. (1960). East African Trypanosomiasis Research Organization Report, January–December, 1959, p. 47. Government Printer, Nairobi.
 Szeinberg, A., Sheba, C., Ramot, B., and Adams, A. (1960). *Clin. Res.*, **8**, 18.

INCREASE IN HAEMOGLOBIN A₂ APPEARING AFTER HOMOGRAFT OF FOETAL HAEMOPOIETIC TISSUE

BY

J. M. BRIDGES, M.D.

D. W. NEILL, M.Sc., F.R.I.C.
Royal Victoria Hospital, Belfast

AND

H. LEHMANN, M.D., Sc.D., M.R.C.P., F.R.I.C.
St. Bartholomew's Hospital, London

The use of chemotherapy for the treatment of cancer has increased greatly in recent years, but as yet no drug has been produced which has a specific action on the malignant process. A factor which often limits the use of these drugs in effective doses is the vulnerability of the bone-marrow to toxic damage by them. If this toxic damage to the haemopoietic system could be efficiently treated then larger doses of the drugs could be employed. The possibility of the use of infusions of haemopoietic cells in the management of this condition has recently aroused much interest. The effectiveness of such treatment will depend on whether the transfused haemopoietic cells mature and are released into the host's circulation.

Two methods are commonly used to determine if cells derived from the transfused material are circulating in the host peripheral blood. The first depends on obtaining the transfused material from a donor of the opposite sex; then, by examining the circulating neutrophils for nuclear appendages, they can be "sexed" and so it can be determined if they arose from host or donor haemopoietic tissue. The second is dependent on obtaining the transfused material from a donor of different blood group from the host; then, by differential agglutination studies of the host peripheral blood, the presence or absence of red cells of the donor group can be demonstrated. Both these methods have technical limitations and also restrict the choice of donor material.

Suspensions of foetal liver contain large numbers of haemopoietic cells. If such foetal material is transfused and the erythropoietic elements survive and mature, then it seems possible that foetal haemoglobin may be produced and circulate in the host's blood. If this were so, then the detection of this haemoglobin would serve as an indication of the maturation of the transfused erythropoietic cells.

The present paper describes our experience in examining the blood of patients for the presence of unusual haemoglobins after use of foetal haemopoietic cell infusions for severe toxic marrow failure.

Eight patients suffering from severe marrow failure subsequent to intensive chemotherapy for various malignant conditions were given transfusions of foetal liver suspension.* The method of collection and storage of this material has been described by Kay and Constandoulakis (1959a). At the time of transfusion all patients were severely ill and showed anaemia, leucopenia, and thrombocytopenia, indicating toxic depression of all facets of marrow function.

In five patients death occurred within nine days of transfusion with no evidence of recovery of haemopoietic function: in none of these patients was any

*The foetal liver suspension was kindly supplied to us by Dr. H. E. M. Kay, of the Royal Marsden Hospital, London.

unusual haemoglobin demonstrated in the peripheral blood.

In the remaining three recovery of marrow function occurred. In every one of them, for a time after the foetal haemopoietic cell transfusion, starch-gel electrophoresis revealed an unusual haemoglobin in the peripheral blood. A short report of each of the three cases follows.

Case 1

A woman aged 44 developed severe marrow failure after a course of thiotepea (triethylene thiophosphoramide) and testosterone for disseminated mammary carcinoma. Her

FIG. 1.—Case 1. Leucocyte and platelet counts during and after chemotherapy and response to foetal liver suspension. (See also Bridges *et al.*, 1960.)

case has already been reported (Bridges, Bridges, Edelstyn, Lyons, and Nelson, 1960). She received 255 mg. of thiotepea over a period of 16 days. A striking clinical and haematological recovery followed transfusion of the foetal cells (Figs. 1 and 2).

On the fourth, seventh, and ninth post-transfusion days examination of the circulating neutrophils for nuclear appendages showed them to be of the

FIG. 2.—Case 1. Haemoglobin level during and after chemotherapy, reticulocyte response to blood and foetal liver suspension, and period during which non-group-A cells and haemoglobin A₂ were present.

male type, but thereafter the circulating neutrophils showed the nuclear appendages characteristic of the female. As the donor material had been obtained from a male foetus these results are interpreted as indicating survival and maturation of the transfused leucopoietic elements.

The donor was blood group O and the host blood group A. Differential agglutination of the circulating red cells on the tenth, thirteenth, sixteenth, and nineteenth days showed non-group-A cells to be present, but after the twenty-second day these could not be demonstrated. The number of non-group-A cells reached a peak on the sixteenth day. These results are taken to indicate that red cells derived from erythropoietic elements of the transfused material were circu-

lating in the host's blood. When the transfused cells ceased to be present in the host peripheral blood spontaneous recovery of marrow function ensued. This patient's malignant disease responded satisfactorily to the anti-cancer chemotherapy and she remains very well 14 months later.

Haemoglobin electrophoresis by the starch-gel method (Smithies, 1955) was done at three-day intervals after the transfusion, and on the first, fourth, and seventh days no abnormal haemoglobin was detected. On the tenth day a slight but definite slow-moving band was present, and this was more definite on the thirteenth, sixteenth, and nineteenth days. It was present but slight on the twenty-second day, but after that time could not be demonstrated. Foetal haemoglobin was measured by the alkali denaturation method (Singer, Chernoff, and Singer, 1951), but on no occasion was the result greater than 2% (normal <2%). Although comparison with other haemoglobin types was not carried out, consideration of Cases 2 and 3 subsequently made it clear that the slow-moving band was most probably haemoglobin A₂.

Case 2

This patient, a woman aged 41, also suffered severe marrow failure after thiotepea and testosterone for disseminated mammary carcinoma. Severe peripheral pancytopenia persisted for 16 days after the withdrawal of therapy, at which time she was given an infusion of blood and foetal liver suspension, which was repeated the next day and again five days later. The peripheral blood findings throughout this period are shown in Fig. 3.

After the first two transfusions there was no improvement in the white-cell count, but some three days after the third transfusion this started to rise, and five days later was 3,000/c.mm. Peripheral blood smears were examined daily for polymorphonuclear appendages, as the donor material had been obtained from a male foetus, but on no occasion was the pattern characteristic of the male observed.

FIG. 3.—Case 2. Peripheral blood findings after blood and foetal liver suspension, and period at which A₂ haemoglobin was present.

Both donor and host were blood group O, and although minor differences in the genotype were present the blood transfusions given at the same time as the foetal liver cells confused subsequent genotype results and these investigations were unhelpful.

On the sixteenth, nineteenth, twenty-second, and twenty-fifth days after the first transfusion of foetal cells a slow-moving band in the haemoglobin electrophoresis similar to that noted in Case 1 was present: as in the previous case, no quantitative rise in foetal haemoglobin could be shown. A specimen taken on the twenty-second day after the first transfusion was submitted to further study and found to contain excess haemoglobin A₂; it is assumed that this was the haemoglobin responsible for the slow-moving band noted on the other occasions. Excess haemoglobin A₂ could not be shown in specimens taken after the twenty-fifth day.

Case 3

A woman aged 61 who suffered from carcinoma of the lung was given two intravenous injections of 15 mg. of tretamine (triethanmelamine); the interval between these was 10 days. Seven days after the second injection her white-cell count was 25/c.mm. and the platelet count was 50,000/c.mm.: these values persisted for a further five days, at which time she was given a transfusion of blood and foetal liver suspension. In Fig. 4 the peripheral blood findings subsequent to this procedure are shown.

The delay after transfusion before the leucocyte and platelet count started to improve makes it almost certain that the transfused material did not contribute to these increases. As in Case 2, both donor and host were blood group O and differential agglutination studies were not possible. Specimens of blood taken on the second and fourth post-transfusion days showed no abnormality, but on the seventh (Fig. 5), eleventh, and fourteenth days an increase in haemoglobin A₂ was noted. This could not be detected after the fourteenth day.

The blood from two patients who developed severe marrow failure subsequent to thiotepa and tretamine therapy but who did not receive foetal liver suspension and in whom spontaneous recovery of marrow function

occurred was examined at frequent intervals. Throughout the period of marrow failure and subsequent recovery no unusual haemoglobin could be demonstrated.

Discussion

The haemoglobin A₂ present in the blood of our three patients after the foetal cell infusions could have been derived from any of three sources: (a) blood transfused at the time of the foetal liver cell infusions; (b) host erythropoietic cells; or (c) transfused foetal erythropoietic cells. The first possibility has been eliminated by examining the blood from all donors from whom our patients received blood. In none of these people was an increased amount of haemoglobin A₂ demonstrated.

That the haemoglobin A₂ arose from the host marrow cells appears most unlikely. It was not shown in any of the five patients who died within a week of the foetal tissue infusion, nor did it appear in the blood of two patients with severe marrow failure whose marrow function recovered spontaneously. In all three patients in whom the haemoglobin A₂ was demonstrated it gradually increased, reached a peak, and then gradually fell off. In Case 1 this peak coincided with the most strongly positive differential agglutination result and also with a reticulocyte response of 9% (Fig. 2). As the differential agglutination test became negative, the haemoglobin A₂ disappeared and the reticulocyte count fell, as did the haemoglobin level. These factors are thought to indicate that at 16 days' post-transfusion the transfused erythropoietic elements were active and the second reticulocyte response occurring on the twenty-fifth day is taken to indicate recovering activity of the host erythropoietic cells.

In Cases 2 and 3 the haemoglobin level fell throughout the period that the abnormal haemoglobin was present, and in each case once the haemoglobin level started to rise, due to recovery of the host erythropoietic activity, the abnormal haemoglobin could no longer be demonstrated.

Thus, in all three cases the period at which haemoglobin A₂ was present did not coincide with the period at which host erythropoietic function appeared active, and the pattern in all three cases strongly suggests an origin of the haemoglobin A₂ from the transfused cells. If so, then the foetal cells in an adult environment must have produced haemoglobin A₂. It would be logical to expect that the blood of patients who have received a transfusion of foetal haemopoietic tissue would contain foetal haemoglobin; and this has in fact been seen in two patients with aplastic anaemia following transfusion with foetal material (Kay and Constandoulakis, 1959b). This was attributed to a temporary proliferation of the transfused cells; however, unpublished findings exist of foetal haemoglobin in such patients at a later stage. Our failure to discover foetal haemoglobin and the observation of a raised haemoglobin A₂ level instead requires, therefore, some explanation.

While adult and foetal human haemoglobin (A and F) are fundamentally similar they differ in the configuration of some of the amino-acid chains. Both have in common two α -polypeptide chains per molecule, but the configuration of the second pair is different, being β -chains in haemoglobin A and γ -chains in haemoglobin F. Recently it has been recognized that haemoglobin A₂, the adult haemoglobin variant found in small quantities besides A₁, also contains two α -chains combined in this case with two δ -chains (Stretton and

FIG. 4.—Case 3. Peripheral blood findings after blood and foetal liver suspension, and period at which A₂ haemoglobin was present.

FIG. 5.—Case 3. Electrophoresis of haemoglobin demonstrating a rise of haemoglobin A₂ on the seventh day. Hanging strip paper electrophoresis, Whatman No. 3 paper "for chromatography," and TRIS buffer (Cradock-Watson, Fenton, and Lehmann, 1959). After electrophoresis the strips were dried and photographed unstained.

Ingram, 1960; Muller and Jonxis, 1960). These three haemoglobins can therefore be designated as follows:

It seems that the infant changes progressively on maturing from γ -chain production to β -chain production, so producing more and more haemoglobin A and less and less haemoglobin F. In the normal child virtually no haemoglobin F is formed after the first year of life. Thus the characteristic feature of the adult environment is the formation of $\alpha_2\beta_2$ globin molecules as in haemoglobin A and the inability to form $\alpha_2\gamma_2$ units as in haemoglobin F. If there is any impairment of haemoglobin A formation, the production of foetal haemoglobin may continue in later life. Once a break occurs in γ_2 -sub-unit formation, however, it appears to be irreversible, and if anaemia appears at an age at which foetal haemoglobin formation has ceased the impairment in haemoglobin A production will not be compensated by formation of haemoglobin F. This could explain the fact that in our patients transfused with foetal cells there was no formation of haemoglobin F in spite of immunological and haematological evidence that the transfused cells were multiplying in the recipient adults.

The transfused foetal cells have, however, a relatively lower capacity for haemoglobin A formation, and their tendency would be to produce more F and A_2 as occurs in β -chain thalassaemia. In this classical thalassaemia haemoglobin A ($\alpha_2\beta_2$) formation is suppressed and there is a compensatory increase in F ($\alpha_2\gamma_2$) and A_2 ($\alpha_2\delta_2$). In our patients the situation is, however, different from that obtaining in this congenital disease, since the foetal cells are introduced into the new environment after $\alpha_2\gamma_2$ (haemoglobin F) formation has long ceased there. Thus, with haemoglobin A production decreased and haemoglobin F formation blocked the transfused cells will tend to increase the $\alpha_2\delta_2$ formation—the haemoglobin A_2 detected by electrophoresis.

Should our observations be confirmed in subsequent cases then the demonstration of excess haemoglobin A_2 would serve as a convenient indicator of the survival and maturation of transfused foetal erythropoietic cells. This method would have the advantage that the fate of the transfused erythropoietic cells could be followed in every case and not only in those in which dissimilarity in the blood groups of donor and host was present.

Summary

Eight patients suffering from some form of malignant disease and who developed severe toxic marrow failure after anti-cancer chemotherapy were given transfusions of haemopoietic cells as contained in foetal liver suspension. Three eventually recovered normal marrow function but the remaining five died within a short period of the transfusion. In all those who recovered, besides haemoglobin A, a "slow"-moving band of haemoglobin became visible on starch electrophoresis of the haemoglobin for a time after the foetal cell transfusion. In two cases this haemoglobin was identified as haemoglobin A_2 and in the remaining case it is felt that it is most likely that excess haemoglobin A_2 was also present.

It is postulated that the haemoglobin A_2 arose from transfused erythropoietic cells and a theoretical explanation is offered. It is suggested that the presence or

absence of haemoglobin A_2 would serve as a useful method of determining if transfused foetal erythropoietic cells had survived and matured in the recipient adult.

We thank Professor H. Harris, of King's College, London, for his interest and help in the identification of the raised haemoglobin A_2 in Case 2; Dr. A. R. Lyons and Mr. J. W. Bingham for allowing us to treat their patients; Dr. M. G. Nelson for his interest and advice; and Miss E. Patterson and Mr. D. Boyd for technical assistance. We acknowledge the financial help given to one of us (J. M. B.) by the British Empire Cancer Campaign.

REFERENCES

- Bridges, J. B., Bridges, J. M., Edelstyn, G. J. A., Lyons, A. R., and Nelson, M. G. (1960). *Lancet*, **1**, 629.
Cradock-Watson, J. E., Fenton, J. C. B., and Lehmann, H. (1959). *J. clin. Path.*, **12**, 372.
Kay, H. E. M., and Constandoulakis, M. (1959a). *Brit. med. J.*, **1**, 575.
— (1959b). *Europ. Congr. Haemat. (Lond.)*. In press.
Muller, C. J., and Jonxis, J. H. P. (1960). *Nature (Lond.)*, **188**, 949.
Schroeder, W. A. (1959). *Fortschr. Chem. org. Naturstoffe*, **17**, 371.
Singer, K., Chernoff, A. I., and Singer, L. (1951). *Blood*, **6**, 413.
Smithies, O. (1955). *Biochem. J.*, **61**, 629.
Stretton, A. O. W., and Ingram, V. M. (1960). *Fed. Proc.*, **19**, 343.

PRIMARY MYELOID METAPLASIA

BY

A. J. BOWDLER, M.B., B.Sc., M.R.C.P.
Medical Registrar

AND

T. A. J. PRANKERD, M.D., M.R.C.P.
Consultant Physician

Medical Unit, University College Hospital Medical School,
London

The disorderly proliferation of haemopoietic cells beyond the physiological demands is the principal concept relating a group of conditions which collectively have become known as the "myeloproliferative disorders." Certain common forms, such as polycythaemia vera, myeloid leukaemia, and essential thrombocythaemia, are named and usually recognized from the characteristics of the peripheral blood; they represent the classical diseases of the group. Dameshek (1951) focused attention on the variety of intermediate forms which may occur, and it is now widely accepted that a blood spectrum of overlapping clinicopathological patterns is embraced by the term. That this is so appears to derive from the proliferative process affecting several cell lineages at various stages of their development, and involving them to differing degrees.

The peripheral blood picture has been strongly emphasized in the descriptions of these conditions, but it is really of importance only in so far as it may reflect the underlying abnormality of proliferation in the tissues, for which histology provides more direct, if less quantitative, evidence. Since little is known at present of the factors governing the release of cells from sites of haemopoiesis the histological evidence must take precedence.

The condition commonly known as "myelosclerosis" is one of the most problematical in the group, and the many synonyms which have been summarized by Heller *et al.* (1947) reflect the wide divergence of views regarding its significant features. In this condition it is