possibility receives some support from the finding of virus particles, possible oncogenic, in cells of NZB mice (East *et al.*, 1967). Other immunosuppressive agents might have similar effects.

Woodruff (1968) reported the recurrence of reticulum cell sarcoma in one patient who died 25 months after transplantation, and had received antilymphocyte serum in addition to azathioprine and prednisone. Antilymphocyte serum has been shown to be a potent immunosuppressive agent in animals (Levey and Medawar, 1966 ; Lance and Medawar, 1968), and its use in man has apparently contributed to increased survival rates of transplanted kidneys from live related donors (Starzl *et al.*, 1968). This serum has also been used in experiments designed to test the role of the immune system in limiting the growth of malignant tumours. The results of several experiments are consistent with the theory that the intact immune system does limit the growth of neoplastic cells but do not provide unequivocal proof (Allison and Law, 1968 ; Deodhar *et al.*, 1968 ; Hellman *et al.*, 1968). The finding that the administration of antilymphocyte serum to mice infected with Maloney leukaemogenic virus results in the development of reticulum sarcomas is of special interest (Allison and Law, 1968).

The extensive suggestive evidence that viruses may produce malignant lymphoproliferative disease in the presence of immunological abnormalities has been reviewed by Schwartz and André-Schwartz (1968). Since both our patients had extensive herpes simplex infection for some weeks before death the possible role of this virus in the production of lymphomata deserves consideration. Herpes-like virus has been incriminated in the pathogenesis of lymphomatosis of fowls (Churchill and Biggs, 1967), and another herpes-like virus, EBV, has been found in the cells of Burkitt's lymphoma grown in tissue culture (Epstein *et al.*, 1964). However, since herpes virus infections are common after renal homotransplantation, and in our series of 38 cases has contributed to death in four, it is probable that this infection was associated with prolonged immunosuppressive therapy and coincident with, rather than causative of, the malignant lymphoma.

It is possible that the increased cellular activity of the immune system in response to a transplanted organ would increase the tendency for neoplastic cells to develop by spontaneous mutation. The occurrence of lymphomata in mice undergoing the graft-versus-host reaction would be consistent with this view (Schwartz and Beldotti, 1965).

The mechanism of production of reticulum cell sarcomata in patients after renal homotransplantation is not known, and the cause, which may be multifactorial, can only be speculated about. It seems likely, however, that the use of potent immunosuppressive agents, probably including antilymphocyte serum, will lead to an increased incidence of primary neoplasia, especially malignant lymphoma.

We thank Mr. A. J. Duke and Mr. C. H. Maclaurin for performing the renal homotransplant operations on these patients.

REFERENCES

Allison, A. C., and Law, L. W. (1968). *Proc. Soc. exp. Biol. (N.Y.)*, **127**, 207.
Burnet, F. M. (1967). *Lancet*, **1**, 1171.
Casey, T. P. (1967). *Proc. Univ. Otago med. Sch.*, **45**, 48.
Casey, T. P. (1968a). *Clin. exp. Immunol.*, **3**, 305.
Casey, T. P. (1968b). *Aust. J. exp. Biol. med. Sci.*, **46**, 327.
Churchill, A. E., and Biggs, P. M. (1967). *Nature (Lond.)*, **215**, 528.
Deodhar, S. D., Crile, G., jun., and Schofield, P. F. (1968). *Lancet*, **1**, 168.
Doell, R. G., De Vaux St. Cyr, C., and Grabar, P. (1967). *Int. J. Cancer*, **2**, 103.
Dossetor, J. B. (1965). Unpublished data. Presented at Washington Conference on Human Kidney Transplants.
East, J., Prosser, P. R., Holborow, E. J., and Jaquet, H. (1967). *Lancet* **1**, 755.
Epstein, M. A., Achong, B. G., and Barr, Y. M. (1964). *Lancet*, **1**, 702.
Hellmann, K., Hawkins, R. I., and Whitecross, S. (1968). *Brit. med. J.*, **2**, 533.
Lance, E. M., and Medawar, P. B. (1968). *Lancet*, **1**, 1174.
Lancet, 1968, **1**, 1298.
Levey, R. H., and Medawar, P. B. (1966). *Ann. N.Y. Acad. Sci.*, **129**, 164.
McPhaul, J. J., and McIntosh, D. A. (1965). *New Engl. J. Med.*, **272**, 105.
Martin, D. C., Rubini, M., and Rosen, V. J. (1965). *J. Amer. med. Ass.*, **192**, 752.
Schwartz, R. S., and André-Schwartz, J. (1968). *Ann. Rev. Med.*, **19**, 269.
Schwartz, R. S., and Beldotti, L. (1965). *Science*, **149**, 1511.
Starzl, T. E., Groth, C. G., Terasaki, P. I., Putnam, C. W., Brettschneider, L., and Marchioro, T. L. (1968). *Surg. Gynec. Obstet.*, **126**, 1023.
Swanson, M. A., and Schwartz, R. S. (1967). *New Engl. J. Med.*, **277**, 163.
Wilson, R. E., Hager, E. B., Hampers, C. L., Corson, J. M., Merrill, J. P., and Murray, J. E. (1968). *New Engl. J Med.*, **278**, 479.
Woodruff, M. P. A (1968). *Antibiot. et Chemother. (Basel)*. In press.

FOR DEBATE . . .

Differences between α- and β-Chain Mutants of Human Haemoglobin and between α- and β-Thalassaemia. Possible Duplication of the α-Chain Gene*

H. LEHMANN,† M.D., SC.D., F.R.C.P. ; R. W. CARRELL,‡ M.B., B.SC., PH.D.

Brit. med. J., 1968, **4**, 748–750

Summary: Human adult haemoglobin consists of two unlike pairs of polypeptide chains, and can be described as $\alpha_2\beta_2$. Amino-acid substitutions in either of the two types of chain result in α- and β-chain variants. In thalassaemia, which causes a lowered production of haemoglobin, the α or the β chain can be affected, the result being α- or β-thalassaemia. There is a quantitative difference in the proportion of α- and β-chain variants to normal haemoglobin in the respective heterozygotes, and there is also a difference in the pattern of inheritance of α- and β-thalassaemia: these could possibly be explained by assuming that man has one gene for the β- and two for the α-chain.

Introduction

The globin polypeptide chains of the known animal haemoglobins and of the myoglobins differ considerably in their primary amino-acid sequence, but they resemble each other in their three-dimensional (tertiary) structure. An amino-acid residue of such a chain can be denoted by the number it occupies in the sequence. Normal adult human haemoglobin, Haemoglobin A, is formed by two pairs of unlike chains, α and β,

* From the Medical Research Council Abnormal Haemoglobin Research Unit, University Department of Biochemistry, Cambridge.
† Professor of Clinical Biochemistry, University of Cambridge.
‡ Chemical Pathologist. Present address: Christchurch Hospital, Christchurch, New Zealand.

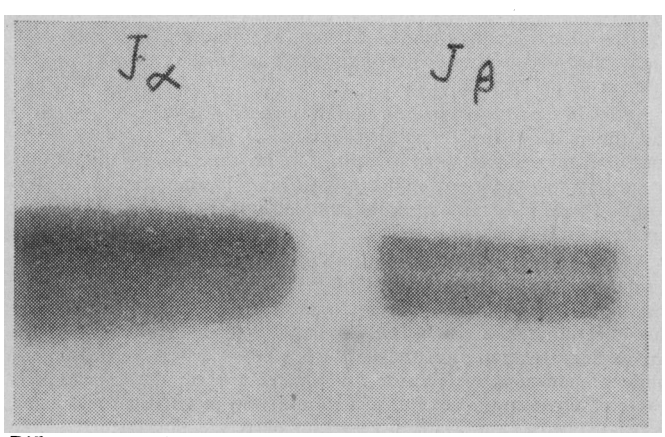
the first consisting of 141 and the second of 146 residues. Haemoglobin M Iwate differs from Haemoglobin A by the substitution of the haem-linked histidine by a tyrosine in the 87th position in the α-chain. This mutation can therefore be described as α87 His → Tyr. The sequential number of the haem-linked histidine in the β-chain is 93. This residue is a tyrosine in Haemoglobin M Hyde Park, and this mutation can be described as β93 His → Tyr. It is important, however, to consider not only the primary structure of the globin chains but also their three-dimensional (tertiary) structure (Watson and Kendrew, 1961 ; Perutz, 1965) It will then be found that, however different in their amino-acid sequence, all myoglobin and haemoglobin chains have the same configuration (Perutz *et al.*, 1965).

Amino-acids can be strung together loosely or they can be linked to compose a firm spiral or helix. The globins consist of eight helical portions A–H, and the interhelical portions are denoted by the letters of the preceding and succeeding helices, all of which may or may not be of the same length in the different globins. The haem-linked histidine is the eighth residue in the F helix, both of the α- and β-chain. Thus by using the nomenclature of the crystallographers M Iwate can be described as α F8 His → Tyr and M Hyde Park as β F8 His → Tyr.

A Possible Explanation

There are now known some 100 variants of human adult Haemoglobin A ($\alpha_2\beta_2$). About one-third are α-chain variants, and two-thirds are β-chain variants (see Perutz and Lehmann, 1968). There are wide differences in heterozygotes of the proportion of the mutant haemoglobin. Those which cause disease in the heterozygous state, such as the unstable haemoglobins which are associated with inclusion body anaemia, are present in small amounts usually about 10–15% whether they are α- or β-chain mutants. With the other haemoglobins there is a notable difference between α- and β-chain abnormal haemoglobins. Unless thalassaemia is also present, the former consistently never amount to more than about 20%, whereas the latter are present at a higher concentration and quite often represent one-half of the total adult haemoglobin. This was related to the possibly greater pathological effect of varying the normal α-chain, particularly because, as noted already, the proportion of severely pathological β-chain abnormal haemoglobins, such as Haemoglobin Köln, is also low (Lehmann *et al.*, 1964).

The substitution of glycine by aspartic acid in the 13th residue of the A helix occurs in the α-chain in Haemoglobin J Oxford, and in the β-chain in Haemoglobin J Baltimore. The first comprises 20% of the total haemoglobin in the heterozygote, but the second amounts to 50% (see Fig.). One cannot exclude that the two glycine → aspartic acid mutations are the outcome of different changes of the codon for glycine, both resulting in aspartic acid but associated with different amounts of messenger R.N.A. However, the same difference in proportion noted on comparing Haemoglobins Jα Oxford and Jβ Baltimore is also found for Haemoglobins α and β E7 His → Tyr (M Boston and M Saskatoon), α and β F8 His → Tyr (M Iwate and M Hyde Park), and α and β GH4 Glu → Lys (O Indonesia and O Arab). These 1:2 proportions we have measured ourselves. There can also be added a comparison between α and β B4 Glu → Lys (Chad and E Saskatoon). For the second we found the variant to amount to 40% in the heterozygote, and for the α-chain mutant, Haemoglobin Chad, Boyer *et al.* (1968) found the percentage to be 15–17%. It is perhaps more likely that the explanation for the different proportions of the A13 α and A13 β Gly → Asp mutants, and the quantitative differences between α- and β-chain mutants in general, might be that man has two pairs of α-chain genes not necessarily resulting in α-chains of different amino-acid sequence, and that in a heterozygote who would then have four α-chain genes, only one would be abnormal.



Different proportions of the mutants α A13 Gly → Asp (Oxford) and β A13 Gly → Asp (Baltimore). The α chain mutant amounts to 20% and the β chain mutant to 50%. (Paper electrophoresis pH 8.9.)

D

Recently two γ-chains have been demonstrated for foetal haemoglobin ($\alpha_2\gamma_2$) where the 136th residue of the γ-chain may be either alanine or glycine (Schroeder *et al.*, 1968), and it is noteworthy that variants of Haemoglobin F amount to about 20% of the total foetal haemoglobin.

In animals duplication of the α-chain is known to occur in the goat (Huisman *et al.*, 1967), and has been suggested for deer (Huisman *et al*, 1968), and what seemed an ambiguity of the genetic code for the α-chain of the rabbit has now been shown to arise from two different genetic processes (Schapira *et al.*, 1968). There are four different α-chains in the horse, where, however, the evidence in favour of allelic genes, though strong, is as yet not conclusive (Kilmartin and Clegg, 1967).

Two pairs of human α-chain genes would also explain why there is such considerable variation of severity of α-chain thalassaemia, in contrast to the position with β-chain thalassaemia. Though different types of β-thalassaemia associated, for example, with raised Haemoglobin A_2 rather than with raised Haemoglobin F and vice versa may well exist, the basic pattern of the mildly but recognizably affected heterozygote and the severely affected homozygote remains. In the case of α-thalassaemia the pattern of severity varies much more widely (Weatherall, 1964 ; Pootrakul *et al.*, 1967). There is one type of α-thalassaemia which can be deduced either from family studies only or sometimes from the presence of traces of Haemoglobin Bart's (γ_4) found at birth. Haemoglobin H disease (Gouttas *et al.*, 1955 ; Rigas *et al.*, 1955) is recognized to be the outcome of more than one abnormal gene (Wasi *et al.*, 1964), but can vary from mildest haematological changes such as occasional cells with Haemoglobin H (β_4) inclusion bodies (Fessas, 1962) to a condition with a clinical severity somewhat greater than that of β-thalassaemia minor. Lastly, α-thalassaemia causes hydrops foetalis where Haemoglobin A ($\alpha_2\beta_2$) and F ($\alpha_2\gamma_2$) are found in traces only, and the majority of the haemoglobin is Bart's (γ_4) and H (β_4) (Lie-Injo Luan Eng *et al.*, 1962).

Two pairs of α-chain genes would explain why α-thalassaemia can be so varied, and why a single α-thalassaemia gene should cause so much less morbidity than a single β-thalassaemia gene. The different degrees of Haemoglobin H disease would then be the outcome of heterozygosity or homozygosity involving two of four α-chain genes, while in hydrops foetalis three of four genes would be affected, and one would expect the doubly homozygous state to be lethal. It has to be admitted, though, that at present some clinical and family studies could be used to support a three α-thalassaemia gene theory for at least a proportion of the patients with Haemoglobin H disease, and a four α-thalassaemia gene status for hydrops foetalis.

References

Boyer, S. H., Crosby, E. F., Fuller, G. F., Ulenurm, L., and Buck, A. A. (1968). *Amer. J. hum. Genet.*, **20**. In press.

Fessas, Ph. (1962). *Haemoglobin Colloquium*, Wien, edited by H. Lehmann and K. Betke, p. 74. Stuttgart.

Gouttas, A., Fessas, P., Tsevrenis, H., and Xefteri, E. (1955). *Sang*, **26**, 911.
Huisman, T. H. J., Wilson, J. B., and Adams, H. R. (1967). *Arch. Biochem.*, **121**, 528.
Huisman, T. H. J., Dozy, A. M., Blunt, M. H., and Hayes, F. A. (1968). *Arch. Biochem.*, **127**, 711.
Kilmartin, J. V., and Clegg, J. B. (1967). *Nature (Lond.)*, **213**, 269.
Lehmann, H., Beale, D., and Boi-Doku, F. S. (1964). *Nature (Lond.)*, **203**, 353.
Lie-Injo Luan Eng, Lie Hong Gie, Ager, J. A. M., and Lehmann, H. (1962). *Brit. J. Haemat.*, **8**, 1.
Perutz, M. F., and Lehmann, H. (1968). *Nature (Lond.)*, **219**, 902.
Perutz, M. F. (1965). *J. molec. Biol.*, **13**, 646.
Perutz, M. F., Kendrew, J. C., and Watson, H. C. (1965). *J. molec. Biol.*, **13**, 669.
Pootrakul, S., Wasi, P., and Na-Nakorn, S. (1967). *Ann. Hum. Genet.*, **31**, 149.
Rigas, D. A., Koler, R. D., and Osgood, E. E. (1955). *Science*, **121**, 372.
Schapira, G., Dreyfus, J. C., and Maleknia, N. (1968). *Biochem. biophys. Res. Commun.*, **32**, 558.
Schroeder, W. A., *et al.* (1968). *Proc. nat. Acad. Sci. (Wash.)*, **60**, 537.
Wasi, P., Na-Nakorn, S., and Suingdumrong, A. (1964). *Nature (Lond.)*, **204**, 907.
Watson, H. C., and Kendrew, J. C. (1961). *Nature (Lond.)*, **190**, 670.
Weatherall, D. (1964). *N.Y. Acad. Sci.*, **119**, 463.

Medical Memoranda

Fulminating Hyperthermia and General Anaesthesia

Brit. med. J., 1968, **4**, 750–751

A paper read by Dr. G. E. S. Relton at the 1967 Belfast meeting of the Association of Anaesthetists stimulated us to look again at the facts of the mysterious deaths of two fit young adults in this region in recent years after minor operations. A number of such occurrences have been published, mainly in Canada (Saidman *et al.*, 1964; Cullen, 1966; Davies and Graves, 1966; Hogg and Renwick, 1966; Lavoie, 1966; Relton *et al.*, 1966; Thut and Davenport, 1966; Purkis *et al.*, 1967; Stephen, 1967), but we know of only one other comparable case so far recorded in the British Isles (Brown, 1954).

Case 1

A 25-year-old man was admitted to hospital in December 1965 after a fall at work. He had sustained a fracture of the right radius at the elbow and a mid-shaft fracture of the left radius. There was nothing of note in the medical history. Physical examination showed a well-built, fit, and muscular young man. Apart from a few abrasions there were no abnormalities other than the injuries mentioned.

He was premedicated with pethidine 100 mg. and atropine 0.6 mg. Anaesthesia was induced with sodium thiopentone 500 mg. followed by suxamethonium 60 mg., and ventilation with 100% O_2, before the passage of a 9.5 cuffed Magill tube. No abnormal response to suxamethonium in the form of increased fasciculation or increased muscular rigidity was noted. Spontaneous respiration returned within a few minutes, and anaesthesia was maintained with nitrous oxide 5 l./min., oxygen 2.5 l./min., and halothane from a Fluotec vaporizer via a Magill circuit.

Closed reduction of the fracture with *x*-ray control was twice attempted but failed. The patient was moved to a theatre for open reduction and fixation. The main operating-theatre formed part of an air-conditioned suite whose air-conditioning was functioning normally.

A tourniquet was applied to the right arm and the operation proceeded. Anaesthesia was not altogether satisfactory. The patient had a persistent tachycardia and a varying respiratory rate. Pethidine 25 mg. intravenously 90 minutes after induction caused transient apnoea, and respiration was "assisted" for four minutes before it was adequate.

As the skin wound was closed, and before plastering, halothane and N_2O was turned off and the patient allowed to breathe pure O_2. At this point the skin was noticed to be "very hot"—a clinical thermometer placed under the tongue registered 110° F. (43.3° C.). Thereafter events moved rapidly, and their sequence is difficult to establish.

All drapes were taken off and cold sponging was begun. At this time extreme rigidity of the skeletal muscles was noted. A second thermometer beneath the tongue registered (by extrapolation) 112° F. (44.4° C.). Two hours had now passed since induction. The patient had become cyanosed and the pulse weak and irregular. Intermittent positive-pressure ventilation with pure oxygen was started by means of a Cyclator. The chest was difficult to inflate, so 30 mg. of tubocurarine chloride was given intravenously, with apparent improvement. While an intravenous saline infusion was being set up the pulse and heart beat disappeared. External cardiac massage was started, and methylamphetamine, dextran, and sodium bicarbonate were given intravenously. One intracardiac injection of adrenaline was given and an external pacemaker tried, but these measures failed to restore the heart beat. The chest was therefore opened, cardiac massage continued, and further intracardiac adrenaline given. Pacemaking by means of direct electrodes was attempted, but despite these measures the heart failed to beat spontaneously. Lack of activity was confirmed by electrocardiography, and death was presumed three hours after induction.

At necropsy both lungs were completely collapsed, and massive collapse of lungs was accepted as the cause of death, though the left lung, at least, could be seen inflating regularly when the chest was opened for cardiac massage. There was no evidence of pulmonary embolism or oedema; the brain showed anoxic changes only, while the heart, kidneys, stomach, and gastrointestinal tract were normal. No specimens of skeletal muscle were examined, nor, regrettably, were blood samples taken during resuscitation for electrolyte and acid–base estimations.

Case 2

This patient, a young woman aged 19, was premedicated with pethidine 50 mg. and atropine 0.6 mg. at 7.45 a.m., before undergoing rhinoplasty. At 8.30 a.m. local application of Brompton cocaine 4 ml. to the nostrils was made, the Moffatt technique being used. Owing to a delay an hour elapsed before induction of anaesthesia was begun, during which time she felt well and her pulse and blood pressure remained within normal limits. At 9.30 a.m. anaesthesia was induced with 2.5% thiopentone 250 mg. and suxamethonium 50 mg. to facilitate oral intubation. Anaesthesia was maintained with 5 litres of N_2O, 2 litres of O_2, and, after spontaneous respiration had returned, by the addition of 2% halothane. During the hour taken to perform the operation the patient's condition was excellent, the pulse rate remaining between 68 and 76, and the blood pressure about 90 mm. Hg systolic. She was in a slight head-up position, and operative conditions were said by the surgeon to be very good.

At 10.30 a.m., at the conclusion of surgery, the tube was removed and the patient conducted to the recovery room; her colour was good and all reflexes were present. At no time during anaesthesia or surgery was there any difficulty with muscle tone or with respiration. There was no abnormal reaction to the induction dose of suxamethonium.

As the patient's level of anaesthesia lightened she started shivering uncontrollably and complained of feeling cold. When the anaesthetist was called to the recovery room to see her the nurse remarked that the skin felt hot. It was observed that she was very flushed. An axillary temperature of 105° F. (40.6° C.) was recorded, and while preparations were made for surface cooling the thermometer was reinserted in the axilla. Within a few seconds cardiac arrest associated with cyanosis and cessation of respiration occurred. At the start of the usual resuscitative measures the thermometer registered 111° F. (43.9° C.) (the mercury was above the top of the