bile-ducts were found at operation, but subsequently the jaundice cleared and the babies recovered. Otherwise the only course open is a minute examination of the anatomy from the portal fissure down to the duodenum through an adequate incision in the hope that a sufficiently dilated piece of hepatic or common duct may be discovered that can be anastomosed to the intestine. If the block is below the entry of the cystic duct and the end of the common duct is very small, the gall-bladder itself can be used, and this is of course technically much easier. If the ducts appear tiny and fibrous, it is worth trying to distend them by injecting through the gall-bladder, if it is present, either saline or methylene blue. Alternatively, diodone may be used and radiographs taken on the table. Such a procedure would obviously be that of choice in those cases with tiny ducts blocked with solid material which can be washed through.

Summary

Four cases of congenital atresia of the bile-ducts are described in which operation for relief of obstruction was attempted, with a successful outcome in one. Microscopical examination of the liver before and three years after operation in the successful case showed complete recovery, with regeneration of damaged liver regions, restoration of biliary flow, and removal of fibrous bands that had begun to grow around the liver lobules.

The clinical and pathological changes produced by congenital atresia are discussed in terms of embryology, and suggestions are put forward for a rational treatment of the condition.

We are grateful to Dr. G. R. Osborn, pathologist to the Derbyshire Royal Infirmary, for the photomicrographs.

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A recent British Standard (B.S. 3277:1960) has been prepared at the request of the Ministry of Health. It deals with tuber-bearing thigh corsets (bucket tops) made of blocked leather (sometimes called moulded leather), non-tuber-bearing thigh corsets of blocked leather, and cuff tops. It distinguishes clearly the essential differences between the three types of appliances and ensures a reasonable quality for the finished product. The standard deals primarily with materials and those details of construction that are independent of individual clinical requirements. Copies may be obtained from the British Standards Institution, Sales Branch, 2 Park Street, London W.1, price 3s., postage extra to non-subscribers.

FOUR HAEMOGLOBINS IN ONE INDIVIDUAL

A STUDY OF THE GENETIC INTERACTION OF Hb-G AND Hb-C

BY

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[WITH SPECIAL PLATE]

Hitherto the finding of a variant of adult haemoglobin together with the normal pigment in any individual has been taken to indicate the presence of a single abnormal gene, and this hypothesis has been confirmed (except for haemoglobin H) whenever adequate family studies have been made. In those instances where two abnormal, but different, genes for haemoglobin production have been inherited one of two results has been found: either the two variants appear without haemoglobin A (as in sickle-cell–haemoglobin-C disease), in which event the genes for the variants concerned can be regarded as two allelomorphs of a normal gene, which they entirely supplant; or the two variants appear together with haemoglobin A (as found, for example, in the simultaneous occurrence of haemoglobins A, D, and K) (Cabannes and Portier, 1959), when one locus no longer suffices. The view that the difference between normal and an abnormal haemoglobin is determined by a single gene has now been clarified by the work of Hunt and Ingram (1959), who have shown that certain of the abnormal haemoglobins differ from normal adult haemoglobin in the chemical structure of only one of the two types of polypeptide chain of which the haemoglobin molecule is composed. Thus the presence of a single abnormal gene is finally seen as a chemical difference in a single polypeptide chain. Recently Itano, Singer, and Robinson (1959) have suggested that in certain circumstances an abnormal haemoglobin may be formed which differs from normal adult haemoglobin not in just one but in both polypeptide chains, and, furthermore, that when this occurs the individual would be expected to possess four major adult haemoglobins. Their argument depends on a consideration of the composition and interaction of the haemoglobin variants.

Human adult haemoglobin (Hb-A) consists of two pairs of polypeptide chains, designated the α^A and β^A chains, with a haem group attached to each chain (Rhinesmith *et al.*, 1958; Perutz *et al.*, 1960), and its molecular formula may be written $\alpha_2{}^A\beta_2{}^A$. The abnormal haemoglobin S (Hb-S) differs from Hb-A in the amino-acid composition of the β-chains, one glutamic-acid residue being replaced by a valine residue, but not in the composition of the α-chains, and thus its formula may be written $\alpha_2{}^A\beta_2{}^S$ (Ingram, 1959). Other abnormal haemoglobins have the same β-chains as Hb-A, but differ in the α-chains. For example, Murayama and Ingram (1959) have shown that in haemoglobin I (Hb-I) the α-chains are abnormal, and

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Itano and Robinson (1959) have given its composition as $\alpha_2^I\beta_2^A$. The substitution, as in Hb–S, of one amino-acid residue for another having a different charge alters the net charge on the haemoglobin molecule and as a consequence alters its rate of migration in an electric field.

At acid *p*H the haemoglobin molecule dissociates into two unlike subunits, which for Hb–A are α_2^A and β_2^A, for Hb–S α_2^A and β_2^S, and for Hb–I α_2^I and β_2^A. When the acid solution is neutralized the subunits combine to re-form the complete haemoglobin molecule. If two different haemoglobins each with a β-chain anomaly are present in the solution subjected to dissociation, then, because there is only one type of α_2 subunit available, recombination can only produce the original two haemoglobins. On the other hand, if the solution contains one haemoglobin with abnormal α-chains and a second with abnormal β-chains, two new hybrid haemoglobins may be formed (Itano *et al.*, 1959). Thus, in the case of Hb–S and Hb–I the two possible "hybrids" are the doubly abnormal haemoglobin I/S ($\alpha_2^I\beta_2^S$) and Hb–A, as illustrated by the following equation:

$$\underset{\text{Hb-S}}{2\alpha_2^A\beta_2^S} + \underset{\text{Hb-I}}{2\alpha_2^I\beta_2^A} \xrightarrow{\text{dissociation}} 2\alpha_2^A + 2\beta_2^S + 2\alpha_2^I + 2\beta_2^A$$

$$\xrightarrow{\text{recombination}} \underset{\text{Hb-S}}{\alpha_2^A\beta_2^S} + \underset{\text{Hb-A}}{\alpha_2^A\beta_2^A} + \underset{\text{Hb-I/S}}{\alpha_2^I\beta_2^S} + \underset{\text{Hb-I}}{\alpha_2^I\beta_2^A}$$

Thus four haemoglobin species could be produced, the doubly abnormal haemoglobin I/S being composed of the abnormal subunits of Hb–I and Hb–S.

Itano *et al.* (1959) suggest that the same series of recombinations of subunits may occur *in vivo*, and they believe this to have occurred in the family described by Smith and Torbert (1958), where Hb–S and another abnormal haemoglobin, Hopkins–2 (Hb–Ho.2), segregate independently, and in which some members show the three haemoglobins A, S, and Ho.2 by conventional electrophoretic techniques. Itano *et al.* (1959) have shown that Hb–Ho.2 has abnormal α-chains, and they have therefore postulated that in those members who show three haemoglobins the haemoglobin migrating in the position of Hb–A is actually a mixture of the doubly abnormal haemoglobin Ho.2/S ($\alpha_2^{Ho.2}\beta_2^S$) and Hb–A. The doubly abnormal haemoglobin in this instance has the same charge, and therefore the same mobility, as Hb–A, because the extra positive charges on the β_2^S subunit compared with β_2^A are balanced by the extra negative charges on the subunit $\alpha_2^{Ho.2}$. Its separate existence has not yet been demonstrated, and in this particular instance confirmation of the theory awaits the detailed chemical examination of the "Hb–A" from these cases (Itano *et al.*, 1959).

We report here a study of a family which confirms the postulate that doubly abnormal haemoglobins form *in vivo*. In this family haemoglobin G (Hb–G) with abnormal α-chains, and haemoglobin C (Hb–C) with abnormal β-chains, segregate independently. Two of its members possess four major haemoglobins, which are Hb–A, Hb–G, Hb–C, and the doubly abnormal haemoglobin G/C ($\alpha_2^G\beta_2^C$), the last resulting from the combination of the abnormal subunits of Hb–G and Hb–C.

Methods

Haematological examination was by standard methods.

Foetal haemoglobin was estimated by the method of Singer *et al.* (1951).

Electrophoresis on cellulose acetate was as described by Kohn (1957), using diethyl barbiturate buffer of *p*H 8.6 and ionic strength 0.05.

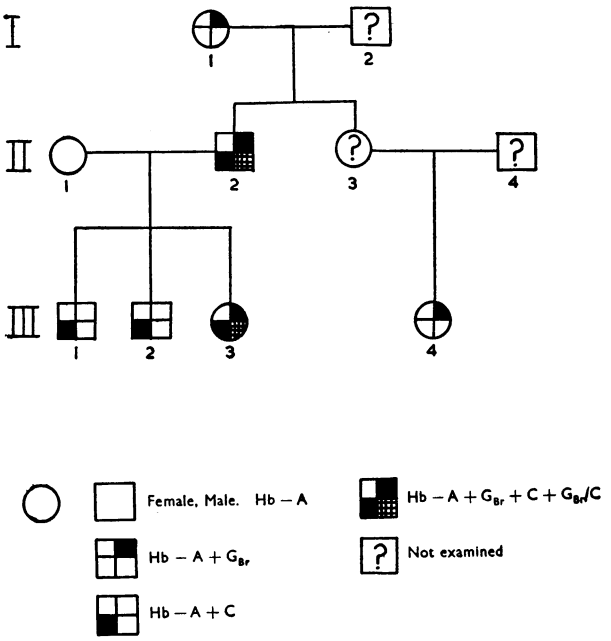
Electrophoresis on starch block followed the method of Kunkel (1954), and *in starch gel* the method of Smithies (1955) using either the discontinuous buffer system (Poulik, 1957) or a phosphate buffer system (Huehns *et al.*, 1960). The haemoglobin zones in starch gel were detected with *o*-dianisidine (Owen *et al.*, 1958). After separation of the haemoglobin zones on starch block the appropriate zone was cut out and the haemoglobin eluted from the starch with 0.02M sodium chloride. The haemoglobin was reconverted to the carbonmonoxy form, its concentration determined by measuring the optical density at 570 mμ, and the solution subsequently concentrated in $\frac{1}{4}$-in. (6.4-mm.) dialysis tubing *in vacuo* in the cold, being simultaneously dialysed against 0.02M saline.

Chromatography on the ion-exchange resin IRC 50 followed the procedure of Huisman and Prins (1955).

Hybridization of haemoglobins was carried out according to the principle described by Itano and Singer (1958), but on the microscale devised by Gammack *et al.* (1960).

Family Studies

The propositus (III-3 on accompanying family tree) was a female child, aged 1 year, of immigrant Barbadoan negro parents, who presented with an upper respiratory



The family tree.

tract infection. No abnormal physical signs were found except those of the above infection. Liver and spleen could not be felt. She was found to have an iron-deficiency anaemia; haemoglobin 10.4 g. per 100 ml., and serum iron 24 μg. per 100 ml. (see Table). The blood film showed red cells with moderate anisocytosis, hypochromia, and some target cells. Electrophoresis of a haemolysate on cellulose acetate showed four equally spaced haemoglobin bands. Three of these were identified as Hb–A, Hb–G, and Hb–C; the fourth was distinctly slower than Hb–C. The child was treated with iron by mouth for a period of two months, during which no significant rise in the total haemoglobin concentration

occurred; however, some fully haemoglobinized cells were visible in the blood film at the end of this period.

The unique appearance of four haemoglobins in one person led to the investigation of the family (see figure). The father (II-2) was also found to have the same four haemoglobins (Special Plate, Fig. 1*d*). He was healthy and there was no history of anaemia. The spleen could not be felt. His haemoglobin was 17.8 g. per 100 ml. and his blood film appeared normal (see Table). The mother carried only Hb–A (Special Plate, Fig. 1*c*) and was haematologically normal (see Table). The red cells from these subjects did not sickle. Other members of the family had remained in Barbados, but with the co-operation of Drs. J. MacIver and L. E. Went and their colleagues blood was obtained from two brothers (III-1 and III-2) of the propositus, her paternal grandmother (I-1), and a female cousin (III-4). Drs. MacIver and Went have shown that III-1 and III-2 both carry the Hb–C trait, while subsequent investigation in Britain revealed that I-1 and III-4 both carry the Hb–G trait (Special Plate, Fig. 1*a* and *b*). In all cases the percentage of foetal haemoglobin was within normal limits.

Experimental Studies

Identification of Haemoglobins

Electrophoresis of the haemolysate of the propositus of her father on both cellulose acetate and in starch gel (Special Plate, Fig. 1*d* and *e*) showed four haemoglobin bands, one migrating in the position of Hb–A, one slightly faster than Hb–S, one in the position of Hb–C, and one slower than Hb–C. The minor haemoglobin band migrating in front of Hb–A in the haemolysates of II-2 and III-3 as well as of II-1 is Hb–A_3, which is often found in association with Hb–A and is presumed to be derived from it (Kunkel and Bearn, 1957). The same four major bands appeared on starch block, and the identity of the faster three zones with Hb–A, Hb–G, and Hb–C was confirmed by elution of these zones and subsequent chromatography on IRC 50 resin (Special Plate, Fig. 2). The fourth zone was similar on chromatography to Hb–C. In addition, the haemoglobin in the third and fourth zones was rapidly converted from carbonmonoxyhaemoglobin to methaemoglobin, a characteristic property of Hb–C. In order to distinguish the haemoglobin G in this paper from those previously reported (Edington *et al.*, 1955; Schwartz *et al.*, 1957; Gammack *et al.*, 1960) it will be called haemoglobin $G_{Bristol}$(Hb–G_{Br}).

The proportions of the four haemoglobins in II-2 determined after elution of the separated zones from starch block were 43%, 23%, 23%, and 11% of the total haemoglobin for Hb–A, Hb–G_{Br}, Hb–C, and the slowest haemoglobin, respectively. The composition of the haemolysate of the propositus (III–3) appeared from the starch-gel analyses (Special Plate, Fig. 1*d* and *e*) to be similar. The Hb–G_{Br}-trait III-4 had 55% Hb–A and 45% Hb–G_{Br}.

Hybridization Experiments

Dissociation and recombination of a solution containing the two haemoglobins G_{Br} and C from II-2 produced two new haemoglobin species migrating in the positions of the fastest and slowest haemoglobin zones of II-2 (Special Plate, Fig. 3*c*, *d*, and *e*). Similarly, after hybridization of the haemoglobin in the fastest (Hb–A) and slowest zones, two additional haemoglobin bands appeared in positions corresponding to the intermediate haemoglobins G_{Br} and C (Special Plate, Fig. 3*a*, *b*, and *c*). In this latter experiment the original solution of the slowest haemoglobin was contaminated with the next fastest haemoglobin, Hb–C, but the increase in Hb–C as well as the new appearance of Hb–G_{Br} can be seen clearly in the figure. In contrast to these results neither a new haemoglobin species nor an increase in any existing species was found when the slowest haemoglobin fraction was hybridized with either of the two intermediate fractions, Hb–G_{Br} or Hb–C (Special Plate, Fig. 4*d*, *e*, and *a*, *b*). The Hb–C and Hb–G_{Br}/C zones in the artificial mixtures of these experiments migrated rather more slowly than the corresponding zones in the haemolysate. A displacement effect of this sort is often observed, however, when the concentrations of the individual haemoglobins in the various solutions being analysed are not identical (Butler *et al.*, 1960).

Discussion

Composition of the Slowest Migrating Haemoglobin

It is clear from the family study that the Hb–G_{Br} and Hb–C in this family segregate independently. The appearance of new species in the experimental hybridization of Hb–G_{Br} with Hb–C (Special Plate, Fig. 3*d* and *e*) suggests that one of these haemoglobins has abnormal α-chains and the other abnormal β-chains. Since Hb–C has been shown to have abnormal β-chains (Hunt and Ingram, 1959), then the Hb–G_{Br} in this family must be abnormal in the α-chains. This allocation of chain abnormality has been confirmed by separate experiments with the Hb–G_{Br} and Hb–C. Thus, when Hb–G_{Br} was hybridized with Hb–S, a variant known to have abnormal β-chains (Hunt and Ingram, 1959), a new haemoglobin species appeared near the position of Hb–C and a zone of increased density appeared in the position of Hb–A (Special Plate, Fig. 5). The latter zone is partially obscured by the foetal haemoglobin present in the specimen from a case of sickle-cell anaemia used in this experiment and by the Hb–A contaminating the eluted Hb–G_{Br}, both of which produce the diffuse zone in front of the GS band in the untreated mixture. This experiment, therefore, shows that Hb–G_{Br} has abnormal α-chains. It would appear that this haemoglobin is possibly another example of the Hb–G with abnormal α-chains (haemoglobin G_{Ibadan}) described by Gammack *et al.* (1960). Hybridization of Hb–C with Hb–H, which consists of four β^A-chains (Jones *et al.*, 1959) produced

Table Showing the Haematological Findings in the Propositus and Her Parents

	Age (Years)	Hb (g. per 100 ml.)	Packed Cell Volume (%)	Red Cells (Millions per c.mm.)	M.C.D. (μ)	M.C.V. ($c\mu$)	M.C.H.C. (g. per 100 ml.)	M.C.A.T. (μ)	Reticulocytes (%)	Alk. Res. Hb (%)	Haemoglobin Types	Anisocytosis	Hypochromia	Target Cells
Propositus	1	10·4	31	5·1	7·6	61	33	1·34	2·1	2·2	A+G_{Br}+C +G_{Br} C	+	+	++
Father ..	36	17·8	49	6·4	7·0	78	36	2·0	...	1·0	A+G_{Br}+C +G_{Br}/C	0	0	0
Mother ..	24	14·1	43	4·6	7·0	94	33	2·4	...	0·8	A	tr.	0	0

Hb–A (Special Plate, Fig. 6), from which it can be concluded that Hb–C has normal α-chains, and therefore by inference abnormal β-chains.

$$2\alpha_2^A\beta_2^C + \beta_2^A\beta_2^A \longrightarrow \alpha_2^A\beta_2^C + \alpha_2^A\beta_2^A + (\beta_2^C + \beta_2^A)$$

Hb–C Hb–H Hb–C Hb–A

The fate of the subunits enclosed in brackets in this equation has not been determined.

It is probable, therefore, that the composition of the two new haemoglobins formed in the hybridization of Hb–G_{Br} with Hb–C are $\alpha_2^A\beta_2^A$ (Hb–A) and $\alpha_2^{G_{Br}}\beta_2^C$, a doubly abnormal haemoglobin, now called Hb–G_{Br}/C, which carries the sum of the charges of the parent species HB–G_{Br} and Hb–C.

$$2\alpha_2^{G_{Br}}\beta_2^A + 2\alpha_2^A\beta_2^C \longrightarrow \alpha_2^A\beta_2^A + \alpha_2^{G_{Br}}\beta_2^A + \alpha_2^A\beta_2^C + \alpha_2^{G_{Br}}\beta_2^C$$

Hb–G_{Br} Hb–C Hb–A Hb–G_{Br} Hb–C Hb–G_{Br}/C

Difference in net charge from Hb–A :—

+2 +4 0 +2 +4 +6

It can be seen in Fig. 3 of the Special Plate that these two new species, Hb–A and Hb–G_{Br}/C, migrate at the same rate as the fastest and slowest of the four haemoglobins occurring *in vivo*. This suggests that the slowest haemoglobin of II-2 is identical with the doubly abnormal haemoglobin G_{Br}/C produced in the above *in-vitro* hybridization. If this is so, then dissociation and recombination of the slowest haemoglobin with Hb–A would be expected, as shown in the following equation, to re-form the two singly abnormal haemoglobins, G_{Br} and C, which are found in the haemolysates of the propositus and her father.

$$2\alpha_2^{G_{Br}}\beta_2^C + 2\alpha_2^A\beta_2^A \longrightarrow \alpha_2^A\beta_2^A + \alpha_2^{G_{Br}}\beta_2^A + \alpha_2^A\beta_2^C + \alpha_2^{G_{Br}}\beta_2^C$$

Hb–G_{Br}/C Hb–A Hb–A Hb–G_{Br} Hb–C Hb–G_{Br}/C

The starch-gel analyses of this particular hybridization experiment (Special Plate, Fig. 3*a*, *b*, and *c*) have confirmed that Hb–G_{Br} and Hb–C are indeed re-formed. On the other hand, no change in composition would be expected if the slowest haemoglobin were hybridized with either of the singly abnormal haemoglobins G_{Br} and C, because the latter two have either the same abnormal α_2 or abnormal β_2 subunit as the doubly abnormal haemoglobin G_{Br}/C. Again, this expectation has been confirmed by the experiments illustrated in Special Plate, Fig. 4. Although in both experiments the hybridized mixtures are somewhat less concentrated than the untreated mixtures, because of precipitation of some haemoglobin in the dialysis of the acidified solutions, no real differences can be seen in the composition of the corresponding hybridized and untreated mixtures. It is thus clear that the propositus and her father possess a doubly abnormal haemoglobin, G_{Br}/C, composed of the abnormal α_2^G and β_2^C subunits of Hb–G_{Br} and Hb–C respectively. In addition, they have two singly abnormal haemoglobins, G_{Br} and C, as well as haemoglobin A.

The in vivo Synthesis of the Four Haemoglobins

Since the propositus received genes for both Hb–G_{Br} and Hb–C from her father, they must presumably occur at different loci. This same situation has been found in at least two other families. In the one described by Schwartz *et al.* (1957) the child, by a normal person, of the propositus, who was heterozygous for Hb–S and another type of Hb–G (as well as for thalassaemia), had neither Hb–S nor Hb–G. In the second family (Smith and Torbert, 1958) the child of a normal father and a mother with Hb–S and Hb–Ho.2 had Hb–S, Hb–Ho.2, and a third fraction migrating in the position of Hb–A —the fraction which Itano *et al.* suggest may contain both Hb–A and the doubly abnormal haemoglobin S/Ho.2.

If it is accepted that the synthesis of the α- and β-chains of a haemoglobin molecule are controlled by pairs of genes at different loci, then the genotype for a person with normal adult haemoglobin may be written α^A/α^A, β^A/β^A (Ingram and Stretton, 1959) Since the propositus and her father possess two abnormal haemoglobins, Hb–G_{Br} with abnormal α-chains and Hb–C with abnormal β-chains, determined by genes at different loci, their genotype is $\alpha^A/\alpha^{G_{Br}}$, β^A/β^C. They would therefore be capable of synthesizing four types of chains, the normal α^A- and β^A-chains as well as the abnormal $\alpha^{G_{Br}}$- and β^C-chains. Now, Itano *et al.* (1959) have pointed out that the absence of hybrid haemoglobins of the type $\alpha_2^A\beta^A\beta^C$ (where, in this example, unlike β-chains are paired) both *in vivo* and in the *in-vitro* hybridization experiments means either that a single polypeptide chain once synthesized rapidly combines with another chain of the same type to form α_2 or β_2 subunits, or alternatively that the chains are synthesized in stable identical pairs. In any event, individuals with this abnormal genotype will synthesize the four different types of subunit α_2^A, β_2^A, $\alpha_2^{G_{Br}}$, and β_2^C, and they will then possess all the subunits necessary for the subsequent synthesis of the four complete haemoglobins.

The final stage in the synthesis of the haemoglobin molecule on this hypothesis is the association of α_2 and β_2 subunits. If the α_2 and β_2 subunits have the same affinity for one another irrespective of type, then the amount of each of the four haemoglobins formed will be determined by the numbers of subunits present at any given time—that is, by their relative rates of synthesis. Itano (1957) has suggested that when an abnormal haemoglobin occurs together with Hb–A the proportion of this haemoglobin that appears in the haemolysate is determined by its rate of synthesis relative to that of Hb–A. Since the heterozygotes for Hb–G_{Br} and Hb–C both show less than 50% of the abnormal haemoglobin (the actual figure for the Hb–G_{Br} is 45% and that for Hb–C traits usually falls between 30% and 45%), it can be inferred that the rate of synthesis of each of these variants is less than that of Hb–A. This carries the further implication that the rate of synthesis of the abnormal subunit is less than that of its normal counterpart and is the rate-limiting step in the formation of the abnormal haemoglobin. Therefore, in the propositus and her father the rates at which the normal α_2^A and β_2^A subunits are being synthesized are greater than for either $\alpha_2^{G_{Br}}$ or β_2^C. It follows that at the association step more $\alpha_2^A\beta_2^A$ (Hb–A) will be formed than either $\alpha_2^{G_{Br}}\beta_2^A$ (Hb–G_{Br}) or $\alpha_2^A\beta_2^C$ (Hb–C). However, it is also true that, because of the relative profusion of α_2^A and β_2^A subunits, more of the abnormal $\alpha_2^{G_{Br}}$ and β_2^C subunits will combine with them to form Hb–G_{Br} and Hb–C than for each other to form Hb–G_{Br}/C, and as a consequence the proportion of the latter will be even less than that of Hb–G_{Br} or Hb–C. It will be noted that the actual proportions found in the father—43% Hb–A, 23% Hb–G_{Br}, 23% Hb–C, and 11% Hb–G_{Br}/C—fall in the order anticipated from the above reasoning.

In contrast, it is unlikely that the four haemoglobins would be found in these proportions if each whole haemoglobin molecule was synthesized independently. Its rate of synthesis would in this event be determined by the formation of the subunit with the lower rate of synthesis. In other words, the amount of Hb–G_{Br}/C formed would be determined by the rate of synthesis of the $\alpha_2^{G_{Br}}$ or β_2^C subunit, whichever was the slower, and

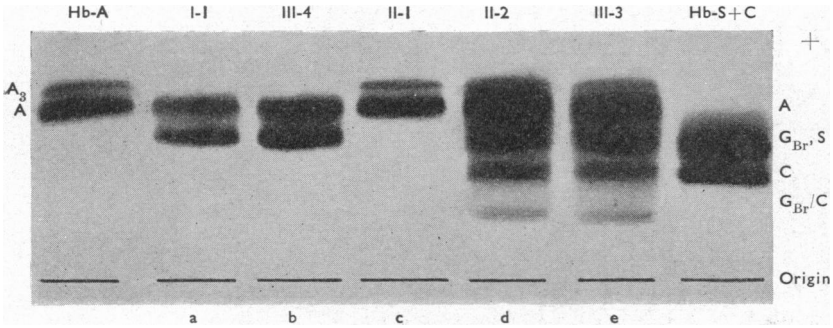


FIG. 1

FIG. 1.—Electrophoresis in starch gel of the haemolysates of some members of the family. Discontinuous tris citrate–borate system.

FIG. 2.—Starch-block electrophoresis of haemolysate of father of propositus (II-2) with subsequent chromatography on IRC 50.

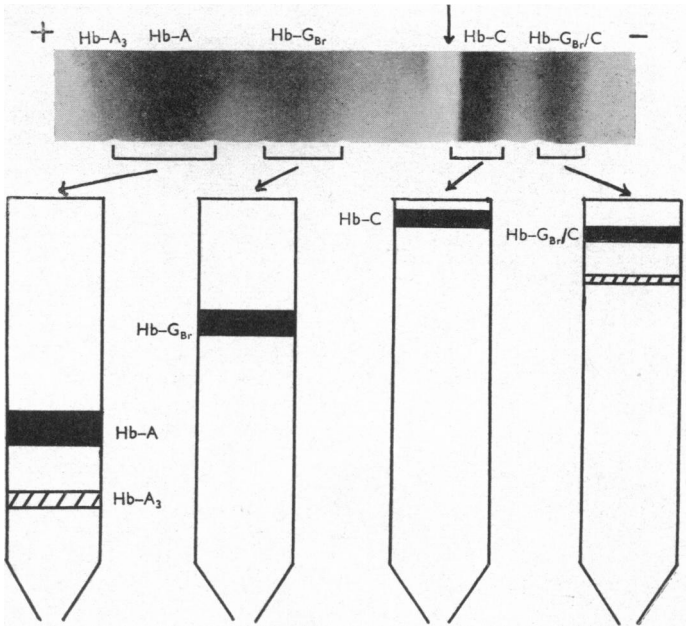


FIG. 2

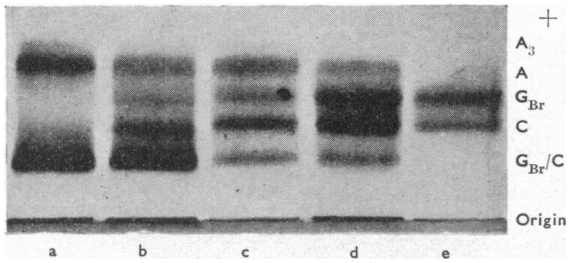


FIG. 3.—Hybridization of isolated haemoglobins from II-2. Electrophoretic analysis in starch gel in the discontinuous tris citrate–borate system. (*a*) Untreated mixture of isolated Hb-A and Hb-G_{Br}/C. (*b*) Mixture of Hb-A and Hb-G_{Br}/C, dissociated and recombined. (*c*) Natural haemolysate from II-2. (*d*) Mixture of Hb-G_{Br} and Hb-C dissociated and recombined. (*e*) Untreated mixture of isolated Hb-G_{Br} and Hb-C.

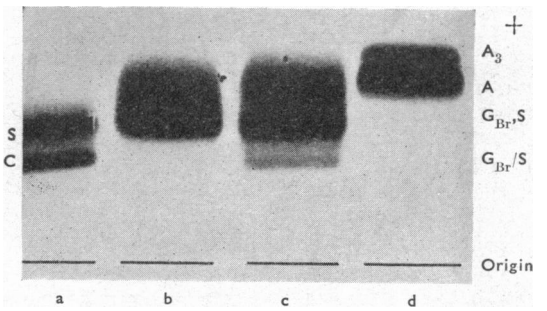


FIG. 5.—Hybridization of isolated Hb-G_{Br} with Hb-S. Electrophoretic analysis in starch gel in the discontinuous tris citrate–borate system. (*a*) Hb-S + Hb-C marker. (*b*) Untreated mixture of Hb-G_{Br} and Hb-S. (*c*) Mixture of Hb-G_{Br} and Hb-S dissociated and recombined. *d*) Hb-A marker.

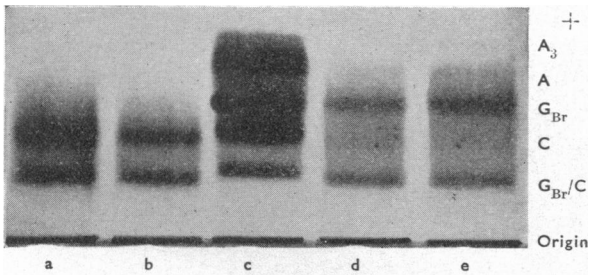


FIG. 4.—Hybridization of isolated haemoglobins from II-2. Electrophoretic analysis in starch gel in the discontinuous tris citrate–borate system. (*a*) Untreated mixture of isolated Hb-C and Hb-G_{Br}/C. (*b*) Mixture of Hb-C and Hb-G_{Br}/C dissociated and recombined. (*c*) Natural haemolysate from II-2. (*d*) Mixture of Hb-G_{Br} and Hb-G_{Br}/C dissociated and recombined. (*e*) Untreated mixture of isolated Hb-G_{Br} and Hb-G_{Br}/C.

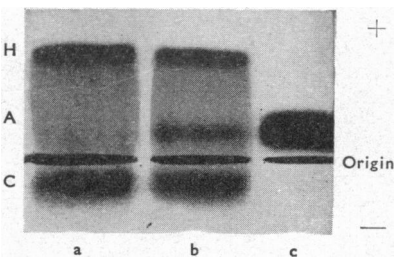


FIG. 6.—Hybridization of isolated Hb-C with Hb-H. Electrophoretic analysis in starch gel in phosphate buffer *p*H 7.4. (*a*) Untreated mixture of Hb-C and Hb-H. (*b*) Mixture of Hb-C and Hb-H dissociated and recombined. (*c*) Hb-A marker.

would finally be equal to the amount of either Hb-G_{Br} or Hb-C. However, in both individuals who possess the four haemoglobins the proportion of Hb-G_{Br}/C is significantly less than that of Hb-G_{Br} or Hb-C.

The further possibility that four haemoglobins could be formed by the synthesis of the two abnormal haemoglobins followed by an intracellular exchange of the subunits has also been raised (Itano *et al.*, 1959). However, on this hypothesis the proportion of Hb-G_{Br}/C would be the same as that of Hb-A, and this again is not so.

The results obtained from the study of this family therefore support the idea that haemoglobin synthesis is controlled by at least two pairs of genes. Furthermore, they suggest that each individual gene finds expression in its own "template," which in turn determines (in effect) the synthesis of a particular type of subunit. These independently synthesized subunits subsequently associate to produce the haemoglobins found in the red cell. If there is a mutation of one gene, as, for example, in the Hb-C trait, the genotype is α^A/α^A, β^A/β^C, resulting in the formation of $\alpha_2{}^A$, $\beta_2{}^A$, and $\beta_2{}^C$ subunits and the subsequent production of Hb-A and Hb-C. When two mutations are present three different situations can arise. First, if the same mutation occurs twice—for example, the genotype is α^A/α^A, β^C/β^C—only the subunits $\alpha_2{}^A$ and $\beta_2{}^C$ of Hb-C are made. On the other hand, when the two mutations are different but occur at the same locus—for example, the genotype is α^A/α^A, β^S/β^C—the subunits $\alpha_2{}^A$, $\beta_2{}^S$, and $\beta_2{}^C$ are made, and this leads to the formation of Hb-S and Hb-C. Finally, if two different mutations occur, one on each locus, as in the propositus of this family—for example, the genotype is $\alpha^A/\alpha^{G_{Br}}$, β^A/β^C—four different types of subunits, $\alpha_2{}^A$, $\alpha_2{}^{G_{Br}}$, $\beta_2{}^A$, and $\beta_2{}^C$, are formed and the four haemoglobins species A, G_{Br}, C, and G_{Br}/C result.

Clinical Features

The propositus had a haematological picture typical of a haemoglobinopathy in that she showed a mild anaemia with target cells in the peripheral-blood smear. Further investigation also revealed concomitant iron deficiency. Her father had the same haemoglobin abnormality, but had no anaemia, and his blood film appeared normal. It seems, therefore, that in this case the anaemia was due to iron deficiency only, and the appearance of target cells was due to haemoglobinopathy and iron deficiency acting together. Her response to oral administration of iron was slow, but one would expect her to reach an almost normal blood picture eventually.

Summary

A family in which two persons carry four haemoglobins—namely, haemoglobins A, G_{Br}, C, and the previously undescribed haemoglobin G_{Br}/C—has been investigated in detail. It has been shown that Hb-G_{Br} and Hb-C segregate independently, and that in the former the α-chains are abnormal and in the latter the β-chains are abnormal. Haemoglobin G_{Br}/C is doubly abnormal, being composed of the abnormal $\alpha_2{}^{G_{Br}}$ subunit of Hb-G_{Br} and the abnormal $\beta_2{}^C$ subunit of Hb-C. The relative proportions of the four haemoglobins found in the propositus suggest that association of the α_2 and β_2 subunits is the final step in haemoglobin synthesis.

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ADDENDUM.—Since this paper went to press, Atwater, Schwartz, and Tocantins have independently described a family in which two members possess the four haemoglobins Hb-A, Hb-G_{Porter}, Hb-C, and a fourth of undetermined composition. These authors point out that, if it is assumed that Hb-G_{Porter} has abnormal α-chains, then the fourth haemoglobin could have the composition $\alpha_2{}^G\beta_2{}^C$.

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The Grenfell Mission provides a medical and social service for the fishermen, Indians, and Eskimos who live along the 2,000-mile coastline of northern Newfoundland and Labrador. The Mission is now using aircraft to bring medical treatment within the reach of everyone for the first time in the history of the country. In the days of travel by boat and dog team many were too isolated to receive even an occasional visit from a doctor or nurse, let alone regular treatment, and patients often reached the mission hospitals too late after days spent in open boats or travelling by dog team. Even to-day, maintaining a medical and social service in a subarctic country is not an easy task, and travel by land, sea, or air is still fraught with dangers and difficulties. Christmas cards are being sold to enable the work of the Mission to be expanded, and a catalogue of these—including one of Grenfell with his huskies—is now obtainable from the Secretary, Grenfell Association of Great Britain and Ireland, Hope House, Great Peter Street, London S.W.1.