Parenteral therapy remains the most reliable method of treating pernicious anaemia, but should this be contra-indicated then generous daily doses of either crystalline vitamin B₁₂ or vitamin-B₁₂-peptide are as effective as any other form of oral therapy yet developed.

I thank the physicians who referred cases to me for these trials; the patients for their co-operation; Dr. J. A. H. Waterhouse for help with the statistical analysis; Dr. J. N. Marshall Chalmers for his co-operation and advice; Mr. C. I. D. Wilson for technical assistance; and Distillers Co. (Biochemicals) Ltd. for supplying the vitamin-B₁₂-peptide.

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Dr. C. S. P. HAMILTON, of Glenageary, Eire, has presented a pewter medal to the Maritime Institute Museum, Dun Laoghaire. The medal was one of a number presented to the crew of the British sloop *Calliope* by the citizens of Apia on the occasion of the hurricane which struck Apia and wrecked several German and American warships in 1889. The *Calliope* was the only ship to sail for the open sea when the hurricane struck. The others threw out extra anchors and attempted to ride out the storm. Some foundered and others were driven ashore. When the *Calliope* returned after the hurricane her Irish commander, Captain Kane, and his crew set to work to bring order out of the destruction in the harbour. In gratitude for their efforts and in admiration of Captain Kane's superb seamanship, the leading residents of Apia gave the captain a silver medal, and each member of the crew a pewter medal. One of these pewter medals, which was given to an Irish sailor, eventually came into the possession of Dr. Hamilton. (*New Zealand News*.)

HAEMOGLOBIN Q-ALPHA-THALASSAEMIA

BY

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Three years ago a new haemoglobin, Q, was described by Vella, Wells, Ager, and Lehmann (1958); it was found in combination with haemoglobin H. Since then there has been much progress in our knowledge of the fine structure of haemoglobin, which we owe largely to Ingram, Schroeder, Itano, and their colleagues. The present paper describes the case of a child in whom haemoglobin Q was found together with haemoglobin Bart's. Taken together with the previous case (Vella *et al.*, 1958), and utilizing the new information on the structure and inheritance of human haemoglobin, our findings make it possible to define a new syndrome—Hb Q- α -thalassaemia.

Case Report

A 10-months-old boy from Thailand, who had developed severe atopic eczema at the age of 7 months, was admitted to the Hospital for Sick Children for treatment. Apart from the eczema, physical examination revealed no abnormal findings; examination of the blood film, however, showed numerous target cells, and further investigations were therefore carried out.

Laboratory Investigations

The results of routine laboratory investigations are summarized in the Table. A stained blood film (Fig. 1, A) showed considerable hypochromia, moderate anisocytosis and poikilocytosis, and many target cells. Except for an eosinophilia, the white cells and platelets were normal. On supervital staining with brilliant cresyl blue a moderate number of red cells were found to contain inclusion bodies (Fig. 2), similar to those found in cells containing haemoglobin H. Red-cell osmotic fragility was greatly diminished, as shown in Fig. 3. The direct Coombs test was negative. The serum bilirubin was 0.2 mg./100 ml. and serum-protein electrophoresis was normal.

The combination of a high serum-iron level with a hypochromic blood picture suggested a diagnosis of thalassaemia. On examination of the haemoglobin by paper electrophoresis at alkaline pH three fractions were observed; the fastest, comprising about 5%, moved in the position of Hb Bart's; the second and major component was found just behind Hb A, in the position of Hb Q; and the third, which amounted to 2% of the whole, was just behind the position of Hb A₂ (Fig. 4). Hb A and Hb A₂ themselves were not found. The finding of Hb Bart's was confirmed by paper electrophoresis at acid pH. On chromatography in "amberlite IRC 50" at pH 6, no Hb A was discovered, and the major component again moved in the position of Hb Q. Hb F was not found, although 2.4% alkali-resistant haemoglobin was shown to be present by the one-minute alkali-denaturation test (Singer, Chernoff, and Singer, 1951). This was explained by the presence of 5% Hb Bart's, which is about half as resistant to alkali as Hb F in this test (Ager and Lehmann, 1958). In several experiments Hb H was not discovered on electrophoresis, although the inclusion bodies (Fig. 2) suggested that some had been present. However, in one experiment with large amounts of very

Results of Routine Laboratory Investigations

Subject	Red Cells (mill./c.mm.)	Hb (g./100 ml.)	P.C.V. (%)	M.C.V. (c. μ)	M.C.H. (μ g.)	M.C.H.C. (%)	M.C.D.* (μ)	Reticulo-cytes (%)	Osmotic Fragility	Inclusion Bodies	Serum Iron (μ g./100 ml.)	Total Iron-binding Capacity (μ g./100 ml.)	Haemoglobins
Propositus	5.55	9.6	35	62.5	31.4	27.4	6.2	5	Greatly reduced	Present	226	350	O_2 , Bart's Q_2 (+H)
Father ..	—	14.4	47	—	—	29.5	6.6	1.4	Slightly reduced	None	62	200	A_2 , Q_2 , A_2
Mother ..	5.0	12.2	41	82	24.4	29.7	6.5	1.8	Reduced	A few	188	320	O_2 , A_2 , A_2 (+H)

* Derived from a Price-Jones curve.

The findings in the father are explained on the basis of iron deficiency, and in the propositus and mother on the basis of thalassaemia.

FIG. 1.—Red cells stained with eosin. ($\times 650$.) A, Propositus. B, Father. C, Mother.

fresh haemolysate, a small trace of haemoglobin was discovered in the position of Hb H; this could only have amounted to much less than 0.5% of the total.

Investigation of the Parents

The father was 34 years old and healthy, and physical examination showed no abnormality. His father had died in old age, and his mother, aged 81, was well; his five sisters and one brother were all alive and well.

The results of routine laboratory investigations are summarized in the Table. The blood film (Fig. 1, B) was normal and no red-cell inclusion bodies were seen in cresyl-blue-stained films. Red-cell osmotic fragility (Fig. 3) was only slightly diminished and the direct Coombs test was negative. The serum bilirubin was 0.1 mg./100 ml. There was no detectable alkali-resistant haemoglobin, and on paper electrophoresis at alkaline pH two major fractions were seen in the positions of Hb A and Hb Q; on staining, these were seen as a confluent broad band (Fig. 4). Hb A₂ was present (2%), and an additional band was seen just behind the Hb A₂, in the position of the abnormal minor

component of the propositus. The slight reduction in both M.C.H.C. and red-cell fragility could be explained on the basis of a mild iron deficiency; the low serum iron is also in favour of this. Further investigation of the father was not possible, however, as he failed to co-operate.

The mother, aged 28, was well; her parents were both alive and healthy, as were her three brothers and four sisters. The stained film showed slight hypochromia, anisocytosis, and poikilocytosis (Fig. 1, C); the white cells and platelets were normal. Films stained supravitaly with brilliant cresyl blue showed a few red-cell inclusion bodies. Red-cell osmotic fragility (Fig. 3) was moderately diminished. The direct Coombs test was negative. Examination of the haemoglobin showed nothing abnormal (Fig. 5), and, although the inclusion bodies had indicated the presence of Hb H, this was not present in sufficient quantities for demonstration by paper electrophoresis.

Discussion

Normal adult haemoglobin (Hb A) consists of two subunits—a pair of α -chains and a pair of β -chains (Rhinesmith, Schroeder, and Pauling, 1957; Rhinesmith, Schroeder, and Martin, 1958); these chains differ in their chemical structure, and it is accepted that they are controlled by different genes. Foetal haemoglobin and haemoglobin A₂ share the α_2 subunits of haemoglobin A, but in combination with γ -chains in Hb F ($\alpha_2\gamma_2$) (Schroeder and Matsuda, 1958; Hunt, 1959), and with δ -chains in Hb A₂ ($\alpha_2\delta_2$) (Stretton and Ingram, 1960; Muller and Jonxis, 1960). The principal

FIG. 2.—Inclusion bodies from Hb H demonstrated by supravital staining with brilliant cresyl blue.

FIG. 3.—Red-cell osmotic fragility (in saline at 20° C.) of propositus, father, and mother. . . . = Father. --- = Mother. -.-.- = Child.

abnormalities of human haemoglobin production are deficiencies of the normal chains, or variations in their chemical constitution. For example, classical thalassaemia can be understood as a deficiency of the β -chain (Ingram and Stretton, 1959), hence in this an increase is seen in $\alpha_2\gamma_2$ (Hb F) and $\alpha_2\delta_2$ (Hb A₂). β -Chain thalassaemia seems to affect the β -chain production only, but not that of the β -chain variants, such as the β_2^S subunits of sickle-cell haemoglobin or the β_2^C subunits of haemoglobin C. Thus in sickle-cell thalassaemia more Hb S is formed than Hb A, and in Hb C disease more Hb C than Hb A.

FIG. 4.—Hanging-strip paper electrophoresis of haemoglobin of father and propositus. Tris buffer pH 8.6. Strips stained with light green. In the father, Hb Q₂ and Hb A₂ are demonstrated; on staining, the Hb A and Hb Q merge into a single broad band. In the child, Hb Q₂, Hb Q, and Hb Bart's are demonstrated. (The uppermost stained band in each strip is not haemoglobin but the protein-X of Derrien *et al.* (1956).)

FIG. 5.—Hanging-strip paper electrophoresis of haemoglobin of mother and propositus. (Preparation as in Fig. 4.) In the mother, Hb A₂ and Hb A are demonstrated; and in the child, Hb Q₂, Hb Q, and Hb Bart's. (The uppermost stained band in each strip is the protein-X of Derrien *et al.* (1956).)

Conversely, α -chain deficiency affects all three normal haemoglobins, and leads to a surplus of β_2 and γ_2 subunits—and presumably also of δ_2 subunits, although this has not yet been demonstrated. The surplus β_2 subunits will combine to form β_4 (Hb H) (Jones, Schroeder, Balog, and Vinograd, 1959), and the surplus γ_2 subunits to form γ_4 (Hb Bart's) (Hunt and Lehmann, 1959). Thus in α -chain thalassaemia a raised Hb A₂ will not be found, and it is noteworthy that Dittmann, Haut, Wintrobe, and Cartwright (1960) found that the relatives of patients with Hb H disease and a thalassaemic blood picture did not show the customary rise in the Hb A₂ fraction. We have therefore two types of thalassaemia: α -chain thalassaemia with Hb Bart's and Hb H, but low Hb A₂ and low or absent Hb F; and β -chain thalassaemia with raised Hb F and raised Hb A₂.

The father of the propositus is a symptom-free haemoglobin Q-trait carrier, and Hb Q is known to have an abnormal α -chain (Jones, Schroeder, and Lehmann; unpublished observations). In the mother a diagnosis of thalassaemia can be made on the basis of a low M.C.H. with a normal serum iron. As neither Hb F nor an increase in Hb A₂ was found, and as a trace of Hb H was probably present, this thalassaemia must be considered to affect the α -chains. The child therefore appears to have inherited Hb Q ($\alpha_2^Q\beta_2$) from the father plus an α -chain thalassaemia from the mother. In other words he has the same condition as that previously described as Hb Q-H disease—except that instead of moderate quantities of Hb H (β_4), Hb Bart's (γ_4) is found with only a very little Hb H, which we now realize has the same significance.

A particularly interesting feature in this case is the abnormal minor fraction, which, following Shooter's example, we may call Q₂ (Shooter, Skinner, Garlick, and Barnicot, 1960). Shooter and his co-workers found that Hb G was the only adult haemoglobin present in a blood sample obtained from West Africa. In this case the abnormal minor component ("G₂") moved as far behind the position of normal A₂ as Hb G moved behind Hb A. It was then found that this particular Hb G (G₁^{Ibadan}) had its abnormality in the α -chain (Gammack, Huehns, Shooter, and Gerald, 1961), and it was concluded that Hb G₂ was a combination of the abnormal

FIG. 6.—Haemoglobin A₂ and haemoglobin Q₂. After discontinuous starch-gel electrophoresis (Poulik, 1957) and staining, the gel was made transparent with glycerol (Gratzer and Beaven, 1960). To obtain the required amounts of A₂ and Q₂, larger quantities of the A+Q mixture had to be used than usual, and the rear part of the A band overlaps with the front part of the Q band.

α_2^G with the normal δ_2 of Hb A₂. The abnormal minor component in our propositus (Q₂) could be explained along the same lines as a combination of α_2^Q and δ_2 —that is $\alpha_2^Q\delta_2$. Further support for this hypothesis is obtained from the observation that the father's haemoglobin contains both A₂ and another minor fraction corresponding to the child's Hb Q₂ (Fig. 6).

Summary and Conclusions

Hb Q- α -thalassaemia was found in a Thai infant. The father is an Hb-Q-trait carrier, and the mother has α -thalassaemia. An interesting feature in the child is the absence of Hb A₂ which is replaced by "Q₂"; in the father A₂ and Q₂ are present together. Taken together with a previously described case of "Hb Q-H disease" the present study establishes a new concept—Hb Q- α -thalassaemia.

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ABO BLOOD GROUPS OF SIBLINGS OF GASTRIC ULCER AND GASTRIC CARCINOMA PATIENTS*

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An increased frequency of blood group O has been found in gastric ulcer patients and an increased group A frequency in gastric carcinoma patients (Buckwalter *et al.*, 1956). Blood donors were used as controls for these studies. Inadvertently, blood donors may be selected from a different stratum of the population than the patients. Differences between the blood type frequencies of the controls and patients might, in this event, reflect anthropological differences between the two strata of the population rather than cause-and-effect association between the ABO blood groups and the

disease in question. By elimination of the possibility of population stratification the siblings of the patients should provide more reliable controls (Clarke *et al.*, 1956). The results of sibship studies support a cause-and-effect explanation for the association between the ABO blood groups and duodenal ulcer (Buckwalter *et al.*, 1960). This is a report of similar studies of siblings of patients with gastric ulcer and gastric carcinoma.

Methods.—The clinical material was provided by patients of the University of Iowa and Iowa City Veterans Administration Hospitals. The diagnosis was based on morphological findings in all of the gastric carcinoma patients and in two-thirds of the gastric ulcer patients; the remainder of the gastric ulcer patients had "ironclad" clinical diagnoses. The methods were the same as those used in the study of duodenal ulcer siblings. The same controls were used. In analysing gastric carcinoma sibships, A₁ and A₂ were regarded as "A" blood, while A₁B and A₂B were regarded as "non-A" blood. The sibships were also analysed after eliminating all sibs differing in sex from their propositi.

Results

The numbers and percentages of the propositi with gastric ulcer, their affected sibs, unaffected sibs, sibs whose ulcer status was unknown, and controls of each ABO blood group are recorded in Table I. Note the increase in group O frequency in propositi (I), as compared with controls (V). When the differences between the blood-type frequencies of the propositi and controls are tested for statistical significance by the chi-square method with three degrees of freedom, $0.02 < P < 0.05$. When all the sibs (II, III, and IV) are combined with the propositi and compared with the controls, $0.02 < P < 0.05$. When the method suggested by Smith is employed (Clarke *et al.*, 1956), which considers each sibship separately, an excess of group O in gastric ulcer propositi (observed) compared with that expected is noted.

In the past, when the Smith method has been used in analysing the data, the working hypothesis has been that a difference between the ABO blood group frequencies of the propositi and sibs might be found. A two-tail test has been used, based on the assumption that this difference might be either an increase or a decrease in O frequency. However, in view of the overwhelming evidence indicating an increase in blood group O frequency in peptic ulcer, and in the light of the work of Clarke *et al.*, an increase in group O frequency is to be expected. If this occurs, then the one-tail test perhaps provides a more valid measure of the significance of the observed differences. The raw data and the P values obtained when the one- and two-tail tests are used in the analysis of the gastric ulcer, and the previously published duodenal ulcer sibship data (Buckwalter *et al.*, 1960) by Smith's method, are recorded in Table II. Note the relatively high level of statistical significance found when the duodenal and gastric ulcer data are combined.

Only two of the gastric ulcer sibships segregated for group O and ulcer after the propositi were excluded. The basic data are included so that other investigators may, if they wish, combine these data with their own in examining the chance of an affected sib being group O with the propositi excluded:

O expected, 0.9286; O observed, 1.0000;
variance, 0.4949.

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