respiratory waves are absent. In Fig. 4, recorded from the same relaxed patient, the airway is only partially obstructed, resulting in an obvious reduction of tidal volume. Fig. 5 shows the effect, again in the same apnoeic patient, of disconnecting the ventilator. This pattern is indistinguishable from that shown in Fig. 4, demonstrating that this technique reveals the essential point—namely, that ventilation of the lungs has ceased. So far as we know this is the only method of monitoring which cannot give a wrong indication in these circumstances.

It is also possible to study the shape of the respiratory wave-form with the impedance spirometer. The tracing follows closely all changes in the volume of air in the lungs, so that it is possible to measure not only the tidal volume but the rate of change of volume, both in inspiration and in expiration. Fig. 6 was recorded on a patient ventilated with a Barnet Mark III ventilator. This machine, though theoretically a constant pressure generator, in practice operates as a constant flow generator (Mapleson, personal communication) and the tracing shows the expected straight line during the inspiratory phase. Fig. 7 was recorded when the same patient was being ventilated with an East–Radcliffe ventilator at approximately the same rate and tidal volume. In this case the tracing shows the exponential curve expected of a constant pressure generator. In both these tracings the expiratory portion is identical, being an exponential decay as the patient expires to atmosphere. The technique may therefore prove helpful in the treatment of asthmatics in whom this decay is prolonged because of the increased lower airways resistance.

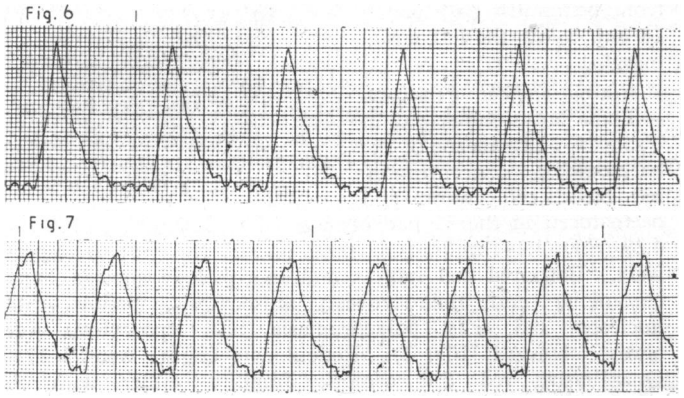


FIG. 6.—Tracing showing the respiratory wave-form when using a Barnet Mark III ventilator. This machine functions as a constant flow generator and the upstroke of the respiratory wave shows the expected straight line. The downstroke, as the patient expires to atmosphere, is exponential. (Paper speed 5 mm./sec.) FIG. 7.—Tracing showing the wave-form when using an East–Radcliffe ventilator to produce the same tidal volume as in Fig. 6. In this case both the upstroke and the downstroke are exponential. This wave-form is typical of a constant pressure generator. (Paper speed 5 mm./sec.)


## Summary

A technique for monitoring respiration is described which is simple to use and simple to understand. Three electrodes are attached to the patient's chest and changes in transthoracic electrical impedance are displayed on an oscilloscope. This tracing reflects the volume of air in the lungs so that the tidal volume, respiratory rate, and respiratory wave-form can be immediately appreciated by those attending the patient.

Additional advantages are that an E.C.G. can be obtained from the same electrodes and that the impedance tracing shows small variations in time with the heart beat. It therefore gives a valuable indication of the function of the heart as well as of respiration. The display provides direct, reasonably accurate, and conceptually simple indications of these two vital factors, important points where nurses are concerned. We feel that this technique is worthy of further trial.



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# Haemoglobin E and α–Thalassaemia*


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A. SUINGDUMRONG,† M.B.


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Haemoglobin Bart's was first described by Ager and Lehmann (1958). The fast-moving haemoglobin earlier reported by Fessas and Papaspyrou (1957) is believed to be identical with Bart's (Fessas, 1959). This haemoglobin has been found by Hunt and Lehmann (1959) to consist entirely of γ-polypeptide chain, thus having the molecular formula of $\gamma_4$; Hbs A, F, and $A_2$ are $\alpha_2\beta_2$, $\alpha_2\gamma_2$, and $\alpha_2\delta_2$ respectively.

Hb Bart's, in extremely variable amount, has been found in conditions ranging from the asymptomatic newborns (Tuchinda *et al.*, 1959; Lie-Injo, 1959; Vella, 1959; Hendrickse *et al.*, 1960; Fessas, 1960; Lie-Injo and Ti, 1961; Schneider and Haggard, 1961; Minnich *et al.*, 1962; Silvestroni and Bianco, 1962; Weatherall, 1963) to the lethal Hb Bart's hydrops foetalis syndrome (Lie-Injo *et al.*, 1962; Banwell and Strickland, 1965; Diamond *et al.*, 1965; Wong, 1965; Pootrakul *et al.*, 1967). Apart from its presence in minute amount in the cord blood of apparently every newborn, the occurrence of Hb Bart's is believed to be a result of α-thalassaemia gene. The latter depresses α-chain synthesis, resulting in excessive γ-chains, which then polymerize to the tetrameric form-$\gamma_4$.

In Thailand Hb E, β-, and α-thalassaemias are prevalent (Na-Nakorn *et al.*, 1956; Flatz *et al.*, 1965; Wasi *et al.*, 1967). These genes, in different combinations, give rise to various conditions and diseases, such as β-thalassaemia homozygosity (Hbs A+F), β-thalassaemia-Hb E disease (Hbs E+F), and Hb H disease (Hbs A+H). Beginning from 1961, we have frequently encountered another disease characterized by the presence of three haemoglobins, A, E, and Bart's. Genetical data indicate that individuals with this disease inherit three abnormal genes—namely, a classical or α-thalassaemia$_1$, a milder

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or $\alpha$-thalassaemia$_2$, and Hb E genes. We wish here to present the clinical and haematological data of this $\alpha$-thalassaemia$_1$/$\alpha$-thalassaemia$_2$-Hb E disease in 21 patients.

## Materials and Methods

Standard haematological techniques were employed. The counting of red blood cells in the latter patients was done in Coulter's electronic counter; quantitative osmotic fragility of the red cells was also switched from the standard method (Dacie, 1960) to Danon's (1963) automatic fragiligraph. Red cell inclusion bodies were demonstrated according to the technique previously described (Na-Nakorn, *et al.*, 1965). The acid elution method of Betke and Kleihauer (1958) was used to demonstrate individual cells containing Hb F.

Haemolysate was prepared according to Singer *et al.* (1951). Originally haemoglobin types were determined by paper and cellulose acetate electrophoreses, and the relative amount of each fraction was obtained by starch block electrophoresis (Kunkel and Wallenius, 1955; Gerald and Diamond, 1958). Later all the haemoglobin typings were done in starch-gel tris-borate-E.D.T.A. buffer, *p*H 8.6 (Smithies, 1959). Haemoglobin fractions were quantitated by electrophoresing on cellulose acetate in tris-borate-E.D.T.A. buffer *p*H 8.6 for one hour, each haemoglobin type being eluted with water from the cut strip, the optical density being read in Beckman B spectrophotometer. Alkali-resistant haemoglobin was measured with the one-minute method (Singer *et al.*, 1951).

## Results

*General and Clinical Description.*—Table I summarizes the general and clinical data. The patients were 5 to 38 years old, those younger than 13 having been seen in the department of medicine only through family investigation. Ten were males. All had Thai ethnic background with mixed Chinese and Mon, each in one instance. They were from central or north-eastern Thailand. Retardation of development was observed in eight cases, being most pronounced in Case 21. "Thalassaemic mongoloid" facies (Fig. 1) was definite in two and suggestive in 12. All were anaemic, and 17 were mildly to moderately jaundiced when first seen. The liver was not palpable in four cases, being 5 to 8 cm. below the right costal margin in the rest. Splenomegaly was noted in all except Case 14, and varied from being just palpable to 18 cm. below the left costal margin.

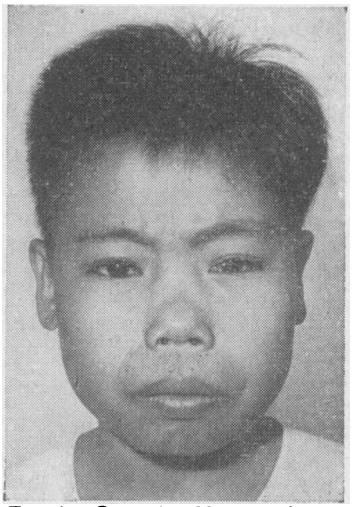

FIG. 1.—Case 21. 22-year-old man showing mongoloid facies.

*Haematological Findings.*—These are summarized in Table II. The means of the haemoglobin concentration, red blood cell count, and haematocrit in the 21 patients were 6.9 (S.D. 1.6) g./100 ml., 4.29 (S.D. 1.14) million/cu. mm., and 27 (S.D. 6) % respectively. Both red cell indices and morphology showed hypochromicity and microcytosis; anisocytosis, poikilocytosis, nucleated red cells, polychromasia, and leptocytosis were always observed (Fig. 2). Reticulocyte counts were 3–17%. Quantitative red cell osmotic fragility, either with the

TABLE I.—*General and Clinical Data*

| Case No. | Age and Sex | Races | Anaemia | Jaundice | Hepatomegaly (cm.) | Splenomegaly (cm.) | Retardation of Development | Mongoloid Facies | X-ray Bone Changes |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 5 F | Central Thai | + | + | 5 | 1 | + | — | Not done |
| 2 | 33 F | N.E. Thai | + | + | 5 | >8 | — | — | — |
| 3 | 37 M | Central Thai | + | + | 5 | 5 | — | — | — |
| 4 | 31 F | " " | + | + | 5 | 5–8 | — | Suggestive | Suggestive |
| 5 | 6 F | " " | + | — | 0 | 5 | + | " | Not done |
| 6 | 22 F | " " | + | + | 0 | 5 | — | — | — |
| 7 | 22 F | " " | + | + | 5 | 5–8 | — | Suggestive | Not done |
| 8 | 38 F | N.E. Thai + Chinese | + | + | 5 | 5 | — | " | — |
| 9 | 17 F | Central Thai + Mon | + | — | 5 | 5–8 | + | — | Suggestive |
| 10 | 28 M | Central Thai | + | + | 5 | >8 | — | Suggestive | — |
| 11 | 17 M | " " | + | + | 5–8 | 5–8 | — | — | Not done |
| 12 | 26 F | N.E. Thai | + | + | 5 | 5 | — | — | " |
| 13 | 16 F | " " | + | + | 5 | 5 | + | Suggestive | — |
| 14 | 9 M | " " | + | — | 0 | 0 | + | " | — |
| 15 | 28 M | Central Thai | + | + | 5 | 5 | + | " | — |
| 16 | 38 M | N.E. Thai | + | + | 5–8 | 8 | — | + | — |
| 17 | 27 M | " " | + | + | 0 | 5 | — | Suggestive | — |
| 18 | 5 M | " " | + | — | 5 | 5 | + | " | — |
| 19 | 34 F | Central Thai | + | + | 5 | 5 | — | " | — |
| 20 | 25 M | " " | + | + | 5 | 5 | — | " | — |
| 21 | 22 M | " " | + | + | 5 | 18 | + | + | + |

TABLE II.—*Haematological Data*

| Case No. | Hb (g./100 ml.) | R.B.C. $\times 10^6$/cu. mm. | Hct % | M.C.V. cu. $\mu$ | M.C.H. $\mu\mu$ | M.C.H.C. % | R.B.C. Morphology | | | | | Retics % | R.B.C. Inclusion % | Hb E % | Hb Bart's % | Alkali Resistant Hb % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Hypo. | Micro. | Anis. | Poik. | Target | | | | | |
| 1 | 6·6 | 4·34 | 27 | 63 | 15 | 24 | +++ | + | ++ | ++ | +++ | 8 | 10 | 14·7 | 11·2 | 3·2 |
| 2 | 8·1 | 4·95 | 34 | 69 | 16 | 24 | +++ | ++ | +++ | +++ | ++ | 10 | 8 | 14·9 | 7·8 | 3·8 |
| 3 | 8·6 | 5·54 | 30 | 55 | 16 | 29 | +++ | ++ | ++ | ++ | ++ | — | — | 13·3 | 8·4 | 2·4 |
| 4 | 4·7 | 2·10 | 18 | 86 | 22 | 26 | +++ | + | + | + | +++ | 9 | 7 | 13·2 | 6·9 | 5·0 |
| 5 | 6·6 | 4·37 | 26 | 60 | 15 | 25 | +++ | +++ | +++ | + | + | 9 | — | 18·7 | 4·8 | 4·0 |
| 6 | 7·5 | 4·47 | 29 | 66 | 17 | 26 | ++ | ++ | ++ | ++ | +++ | 17 | — | 13·6 | 8·3 | 4·8 |
| 7 | 5·7 | 3·35 | 27 | 79 | 17 | 22 | +++ | +++ | +++ | +++ | ++ | — | — | 18·9 | 3·4 | — |
| 8 | 6·6 | 3·76 | 22 | 59 | 18 | 30 | ++ | ++ | ++ | + | + | — | — | 14·5 | 8·0 | 2·5 |
| 9 | 6·7 | 3·79 | 29 | 77 | 18 | 23 | +++ | + | ++ | +++ | + | 6 | 17 | 15·4 | 8·0 | 1·6 |
| 10 | 8·9 | 3·85 | 29 | 75 | 23 | 31 | ++ | ++ | + | + | ++ | 10 | 2 | 18·8 | 4·7 | 2·8 |
| 11 | 5·9 | 4·46 | 26 | 47 | 10 | 20 | +++ | +++ | +++ | +++ | +++ | 4 | 0 | 13·2 | 7·7 | — |
| 12 | 7·0 | 5·21 | 28 | 54 | 13 | 25 | +++ | + | ++ | + | + | 5 | 3 | 14·6 | 7·1 | 4·6 |
| 13 | 4·8 | 3·10 | 18 | 58 | 16 | 27 | ++ | ++ | +++ | ++ | +++ | 3 | 12 | 12·5 | 9·4 | 1·5 |
| 14 | 8·4 | 5·90 | 32 | 55 | 14 | 26 | +++ | ++ | ++ | ++ | +++ | 4 | 16 | 14·7 | 18·0 | — |
| 15 | 7·0 | 4·88 | 29 | 59 | 14 | 24 | ++ | ++ | + | + | ++ | 10 | 5 | 14·5 | 9·2 | 4·7 |
| 16 | 8·4 | 4·43 | 32 | 72 | 19 | 26 | ++ | ++ | ++ | ++ | ++ | 10 | 3 | 13·1 | 8·7 | 4·2 |
| 17 | 9·8 | 6·01 | 39 | 65 | 16 | 25 | ++ | + | ++ | ++ | ++ | 6 | 3 | 13·4 | 8·3 | 4·8 |
| 18 | 7·1 | 5·40 | 23 | 43 | 13 | 31 | +++ | ++ | ++ | ++ | ++ | 10 | 5 | 12·6 | 11·5 | 5·2 |
| 19 | 6·0 | 4·25 | 25 | 59 | 14 | 24 | ++ | ++ | ++ | ++ | ++ | 4 | 10 | 13·6 | 8·6 | 4·1 |
| 20 | 7·2 | 4·61 | 30 | 65 | 16 | 24 | ++ | ++ | ++ | ++ | ++ | 8 | 1 | 12·2 | — | 1·8 |
| 21 | 3·2 | 1·41 | 11 | 78 | 18 | 21 | +++ | +++ | +++ | +++ | +++ | 3 | 2 | 14·4 | 7·0 | 4·2 |

— = not estimated.

standard technique or with Danon's automatic fragiligraph, was constantly decreased. On mixing the blood with methylene blue solution inclusion bodies, unlike those in Hb H disease, were always seen but only in very few red cells (Fig. 3). Acid elution and counterstaining according to Betke's technique showed a few cells retaining haemoglobin (Fig. 4). $^{51}Cr$ red cell half-lives‡ in Cases 7, 9, 13, 16, and 21 were 3.5, 10, 8.5, 12, and 4 days respectively. The bone marrow examination in four cases showed marked erythroid hyperplasia and haemosiderosis.

*Haemoglobin Types.*—The major haemoglobin types in starch gel (Fig. 5) were A+E+Bart's. The level of Hb E fell in a rather narrow range between 13 and 15% in most cases, the extreme values being 12.2 and 18.9%. Hb Bart's constituted 3.4 to 18.0, averaging 8.4%. Alkali-resistant haemoglobin, as determined by Singer's one-minute method, was slightly raised (Table II). A band of presumably Hb F was seen behind that of Hb A in both starch-gel and cellulose acetate electrophoreses; with the latter technique the amounts in Cases 10, 12, 13, 19, and 21 were 4.4, 3.9, 4.0, 3.9, and 7.0% respectively. With both starch-gel and cellulose acetate electrophoreses a faint band of haemoglobin was seen cathodal to Hb E at *p*H 8.6 (Fig. 5). The amount of this slow-moving haemoglobin was 0.53–1.30%. Occasionally a faint band of haemoglobin at Hb H position was observed.

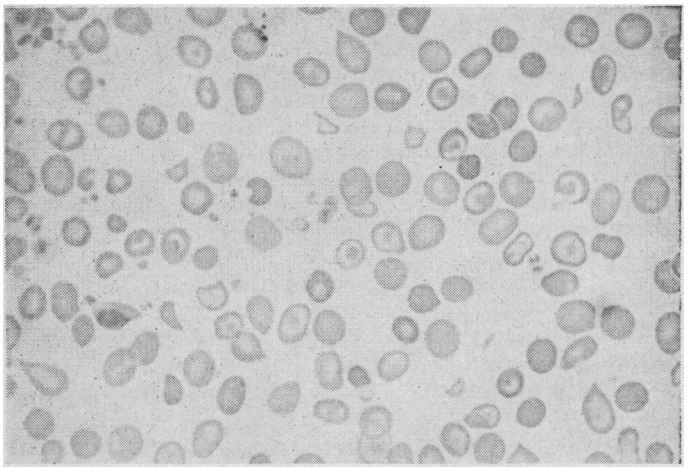

FIG. 2.—Case 16. Peripheral blood smear. (Wright's stained. ×1,280.)

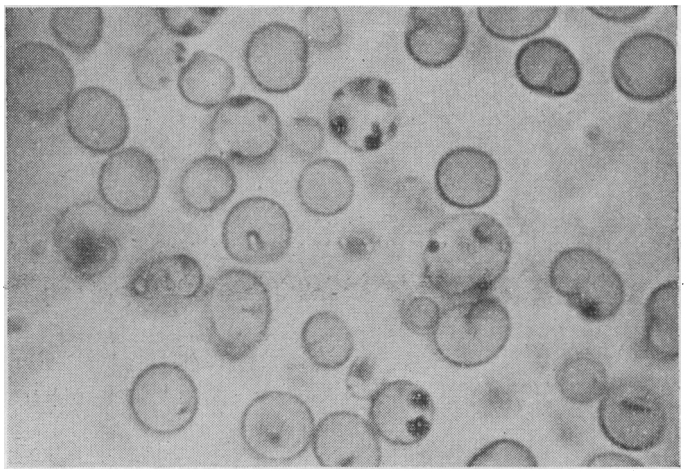

FIG. 3.—Case 18. Methylene blue fresh preparation (×3,200) showing characteristic presence of a few cells with inclusion bodies.

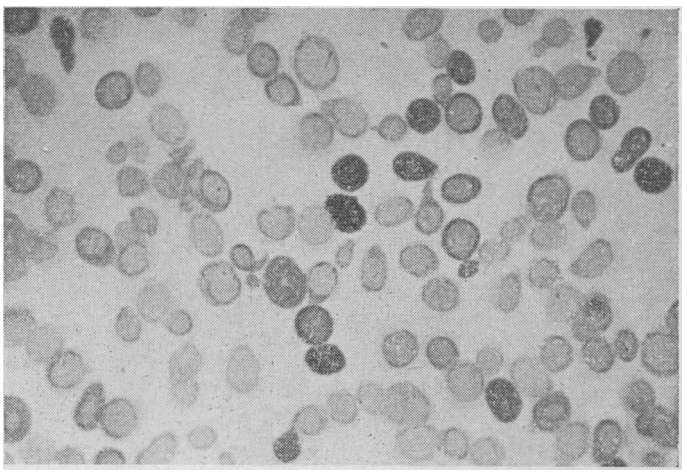

FIG. 4.—Case 21. Acid elution staining (×1,280) showing a few acid-resistant red cells.

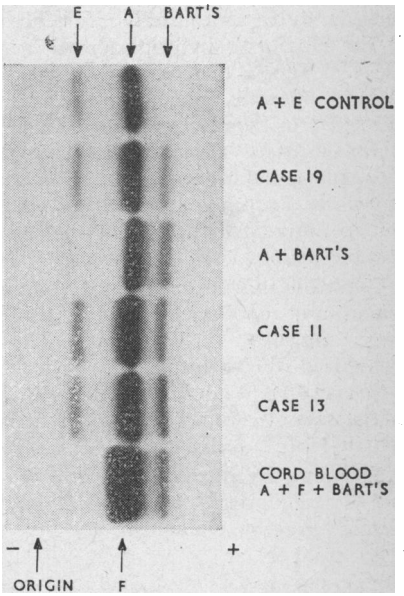


FIG. 5.—Horizontal starch-gel electrophoresis in tris-borate-E.D.T.A. buffer, *p*H 8.6, stained with ortho-dianisidine.

*Other Findings.*—*X*-ray examination of the skulls and long bones in 17 cases showed widening of the medulla typical of thalassaemic diseases in one case, and in two cases the pictures were suggestive. Serum iron was raised in most cases. Conjugated bilirubin level was normal, but the total value was raised up to 5.5 mg./100 ml. Serum globulin was above 4 g./100 ml. in three out of nine patients, and serum electrophoresis showed gammaglobulin of 25% or over in five out of eight cases. Serum flocculation tests were often positive. The fasting blood sugar, serum alkaline phosphatase, transaminases, cholesterol, and sulphobromophthalein excretion, determined in five cases, were within normal limits.

*Clinical Course.*—The patients remained anaemic all the time, though the haemoglobin level may fluctuate considerably. Folic acid orally did not seem to alleviate the anaemia. Hypersplenism was noted in Case 21. Splenectomy had been performed in Cases 2, 3, 4, 10, 14, and 21, with a slight improvement of anaemia in Cases 14 and 21.

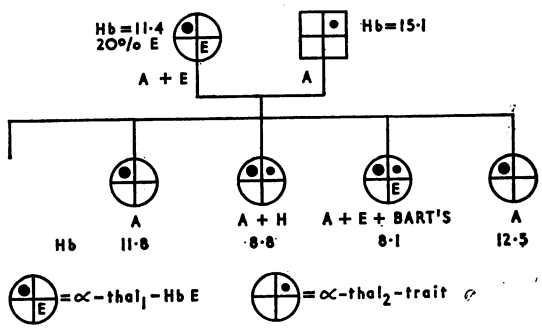


FIG. 6.—Family of Case 2. Detailed genetical data will be described elsewhere.

*Genetical Findings.*—Family members of the patients were examined, but the results are not presented here. Only representative findings from the family of Case 2 are condensed into Fig. 6.

‡ Done by Dr. V. Viranuvatti and others, of the Faculty of Medical Technology.

## Discussion

That this Hb A+E+Bart's disease is connected with thalassaemia is evident from thalassaemic stigmata in the patients themselves and in their parents, siblings, and offspring. This type of thalassaemia is the low Hb $A_2$ or $\alpha$-chain variety of thalassaemia. Two forms of $\alpha$-thalassaemia have been identified in the Thai population (Wasi *et al.*, 1964): $\alpha$-thalassaemia$_1$($\alpha$-thal$_1$) is the classical or the most severe form, and $\alpha$-thalassaemia$_2$($\alpha$-thal$_2$) is the mildest type which cannot be identified in heterozygotes. Those homozygous for the $\alpha$-thalassaemia$_1$ gene die during intrauterine existence with the clinical findings of the Hb Bart's hydrops foetalis syndrome (Pootrakul *et al.*, 1967). The $\alpha$-thal$_1$/$\alpha$-thal$_2$ combination results in Hb H disease. Genetic analysis of the families reported here will be presented in detail elsewhere. They indicate that the patients with Hb A+E+Bart's disease are carrying the Hb E gene and those for $\alpha$-thal$_1$ and $\alpha$-thal$_2$. This condition has been reported as well by Tuchinda *et al.* (1964), who discussed the interaction of two different $\alpha$-thalassaemia genes and Hb E as one of the possible aetiologies.

Clinically this disease cannot be differentiated from $\beta$-thalassaemia homozygosity, $\beta$-thalassaemia-Hb E, and Hb H diseases, much prevalent in Thailand. The severity of the already recognized disease forms appears to be in the following order: $\alpha$-thalassaemia$_1$ homozygosity (Hb Bart's hydrops foetalis), $\beta$-thalassaemia homozygosity, $\beta$-thalassaemia-Hb E, $\alpha$-thal$_1$/$\alpha$-thal$_2$-Hb E, and $\alpha$-thal$_1$/$\alpha$-thal$_2$.

Haemoglobin phenotype of A+E+Bart's is the hallmark of the disease under present discussion. In contrast to Hb H disease, in which inclusion bodies are demonstrable in the majority or all of the red cells, in $\alpha$-thal$_1$/$\alpha$-thal$_2$-Hb E disease only a few red cells show inclusion bodies. The non-overlapping numbers of red cells containing inclusion bodies in these two conditions have led us to correct diagnosis previous to electrophoresis in all instances. These scanty inclusion bodies in the red cells of the patients with Hb A+E+Bart's probably represent Hb H, which is present at too low concentrations to be readily detected by electrophoresis. Hb E, which in heterozygotes is present in about 30% of total haemoglobin, represents about 14% of the total haemoglobin in this disease. Three reasons for the reduced quantity of Hb E in this circumstance may be considered. Firstly, the $\alpha$-thal$_1$/$\alpha$-thal$_2$ combination, in addition to suppressing $\alpha$-chain synthesis, has an inhibitory effect upon Hb E production (Tuchinda *et al.*, 1964). Secondly, unequal distribution of haemoglobin types among red cells may be associated with differential survival as found in Hb H disease (Gabuzda *et al.*, 1965). Finally, $\beta_2$ subunits may compete for $\alpha_2$ subunits more successfully than $\beta_2$E subunits. In view of insufficient data it is not appropriate to discuss these possibilities at length here.

In this disease both Hbs F and Bart's are raised irrespective of the patient's age. The increased amount of alkali-resistant haemoglobin is due to both types of haemoglobin, since Hb Bart's is partially alkali-resistant. According to Betke's technique, cells containing Hb F are relatively resistant to acid elution. In Hb A+E+Bart's disease a few cells are obviously resistant. Whether they represent cells containing only Hb F or Hb Bart's as well is not yet known.

The apparent decrease in $\beta$-chain synthesis in $\alpha$-thalassaemia has been previously discussed (Rucknagel, 1964 ; Pootrakul *et al.*, 1967). As judged by the amount of Hbs F and Bart's found in $\alpha$-thal$_1$/$\alpha$-thal$_2$-Hb E disease the quantity of $\gamma$-chain produced in this condition is obviously greater than that in Hb H disease. This suggests that the amount of $\beta$-chain synthesis in the former is less than in the latter, since the quantity of $\gamma$ and $\beta$ chains is reciprocally related. This would indicate that, for reasons not yet clear, when the Hb E gene is present in addition to the $\alpha$-thal$_1$ and $\alpha$-thal$_2$ genes a reduction in $\beta$-chain synthesis occurs. Thus very little Hb H is found in this disease, despite the $\alpha$-thal$_1$/$\alpha$-thal$_2$ genotype which is identical to that in Hb H disease.

The haemoglobin band slower than Hb E has not yet been identified. Its absence in both parents suggests that it is not a result of primary mutant gene action. It is most likely $\delta_4$, as found in Hb H disease (Dance *et al.*, 1963) and/or $\beta_4$E. Further investigation is in progress.

The patients with this disease seem to benefit only from blood transfusion, and from splenectomy when hypersplenism is present. Liver damage often handicaps the victims. The patients with these various combinations of thalassaemias and Hb E, about 100,000 in number as estimated from gene frequencies, present a socio-economic problem to Thailand.

## Summary

The clinical and haematological study of 21 patients in Thailand with a new disease is described. It is similar to a thalassaemia major disease clinically. Hepatosplenomegaly was almost consistently detected, the mean haemoglobin concentration was 6.9 g./100 ml., the red cells showed strong stigmata of thalassaemia, and the erythrocytic life-span was shortened. This disease is characterized by the presence of inclusion bodies in very few red cells, and haemoglobin phenotype of A+E+Bart's with about 14% Hb E and 8% Hb Bart's. Elevation of Hb F was demonstrated electrophoretically, morphologically, and by the alkali denaturation test. Small amounts of an unidentified haemoglobin slower than E, probably $\delta_4$ and/or $\beta_4$E, were also detected. Genetical data suggest that this disease is a result of a combination of three abnormal genes—namely, the more usual type of $\alpha$-thalassaemia or $\alpha$-thalassaemia$_1$ gene, a milder or $\alpha$-thalassaemia$_2$ gene, and Hb E. Anaemia in these patients was, although fluctuating in degree, perpetual. Splenectomy had been done in six patients, with a slight improvement of anaemia in two—one with hypersplenism.

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