Technical Papers

Hemoglobin E, a Hereditary Abnormality of Human Hemoglobin

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During the course of experiments (*1*) designed to determine why some Thai patients with Mediterranean anemia (*2*) failed to fulfill the genetic criterions postulated for that disease (*3*), an abnormal hemoglobin was detected that had an electrophoretic pattern different from that of other known hemoglobin types (*4–6*). In accordance with recommended nomenclature (*7*), the new pigment was called hemoglobin E. At about the same time, Itano, Bergren, and Sturgeon (*8*) identified an abnormal hemoglobin, which they designated as hemoglobin E, in a child with an atypical anemia. A sample of blood from one of our subjects was sent to them; the two hemoglobins had a similar electrophoretic mobility and are presumably identical (*9*).

We have now found hemoglobin E in the blood of eight Thai subjects. Five of these eight subjects had clinical and hematologic manifestations of Mediterranean anemia; electrophoretic analysis revealed hemoglobin E and hemoglobin F. In four instances it was possible to show that one parent had the Mediterranean trait. The disease may properly, therefore, be called Mediterrean-hemoglobin E disease (*4, 7*). The remaining three subjects were asymptomatic; no specific hematologic abnormalities were recognized. Their blood contained hemoglobins E and A. They are regarded as having the hemoglobin-E trait.

Hemoglobin E was identified by a slight modification of the paper electrophoresis technique described by Smith and Conley (*5*). Electrophoretic runs were made at room temperature for 10 to 14 hr at 350 v and 14 to 16 ma. A sheet of Whatman 3 MM paper, 7.5 in. wide, was placed between siliconized glass plates 8 by 14 by 3/8 in. which were secured together by heavy clamps to prevent puddling of the veronal buffer (*p*H 8.8 and ionic strength 0.06). The paper was marked with a vertical line through the center, soaked in buffer, and blotted almost dry. With a micropipette, 0.003 to 0.005 ml of a 6 to 12 gm percent hemoglobin solution (*10*) was placed on the paper at the center line. Ten specimens could be run simultaneously. The spots could be followed without difficulty as they migrated, and they were clearly visible without staining after the paper had been dried in an oven at 100°C for 30 min. The percentage of fetal hemoglobin was determined by the alkali denaturation technique (*10, 11*).

When hemoglobin solutions are subjected to paper electrophoresis under the conditions outlined, hemoglobin A moves farthest toward the anode in the electric field of the paper, while hemoglobin F moves slightly more slowly and is imperfectly separated from hemoglobin A (*5, 6*). Hemoglobin S migrates still more slowly, and hemoglobin C migrates least of all. The new hemoglobin identified in the Thai subjects moved with a mobility intermediate between that of hemoglobins S and C so that the spot at the end of the run was between those of hemoglobins S and C (Fig. 1). A small quantity of hemoglobin E was prepared relatively free of other types of hemoglobin by elution of the hemoglobin-E spots from filter paper strips. The eluate was dialyzed against four changes of distilled water and analyzed spectrophotometrically in a Beckman spectrophotometer. The absorption spectrum was characteristic of oxyhemoglobin; no deviations from the curve of normal adult hemoglobin in the visible range were detected.

The genetic transmission of hemoglobin E is indicated by a study of the following family in which the father and his three children had hemoglobin E in their blood (Table 1). The mother fulfilled the criterions for the Mediterranean trait: hypochromic polycythemia, a marked decrease in osmotic fragility, and numerous target cells. Electrophoretic study of her hemoglobin showed hemoglobin A, while, by the alkali denaturation test, hemoglobin F was found in normal amounts (*11*). The father was asymptomatic, and he showed minimal hypochromia, a slight decrease in

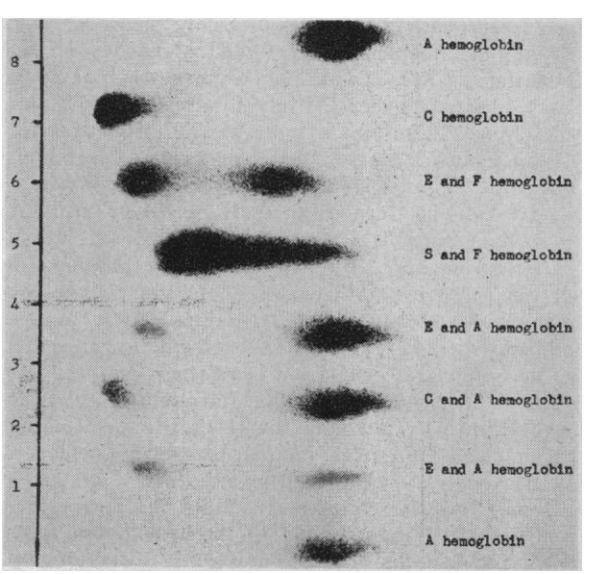

Fig. 1. Paper electrophoresis of a number of hemoglobin specimens containing various types of hemoglobin. Numbers 2 and 4 are examples of hemoglobin-E traits, and No. 6 is an example of Mediterranean-hemoglobin E disease.

Table 1. Hemoglobin components in members of Thai family "K."

Subject	Sex	Age (yr)	Hemoglobin types*			Percentage of hemoglobin F†	Remarks
			E	A	F		
Mrs. K.	F		−	+	−	Normal	Mediterranean trait
Mr. K.	M		+	+	−	Normal	Hemoglobin-E trait
D. K.	F	2½	+	+	+	13.6	Mediterranean-hemoglobin E disease (transfused)
C. K.	M	1	+	−	+	33.0	Mediterranean-hemoglobin E disease
P. K.	F	7	+	+	−	Normal	Hemoglobin-E trait

* By paper electrophoresis method.
† By alkali denaturation technique.

osmotic fragility, and only a few target cells. Electrophoretic analysis of his hemoglobin revealed hemoglobin E in association with hemoglobin A; he was regarded, therefore, as having the hemoglobin-E trait. Two children (D. K. and C. K.) were found to have Mediterranean-hemoglobin E disease, a severe hemolytic anemia, indistinguishable from Mediterranean anemia but with both the E and F types of hemoglobin present. The small amount of hemoglobin A found in D. K. almost certainly resulted from a recent transfusion. Some of the characteristics of the disease are evident in the following data on C. K. This boy has hepatosplenomegaly, and on the day of examination he had 2.24 million erythrocytes per mm^3, 5 g of hemoglobin per 100 ml, 19 percent packed red-cell volume, 8.2 percent reticulocytes, 17,450 leucocytes per mm^3 with a shift to younger forms of granulocytes, and 40 nucleated erythrocytes per 100 white-blood cells. The third child, P. K., is an example of the hemoglobin-E trait.

Of the remaining four subjects who were found to have hemoglobin E, three were unrelated, while the fourth subject was the maternal half-sister of the children listed in Table 1; she presumably inherited the hemoglobin-E gene from her father who was not available for study. One of the four subjects had the hemoglobin-E trait, while the other three, including the maternal half-sister, had Mediterranean-hemoglobin E disease.

Among the cases so far encountered, it is interesting to note that, if the intensity of the hemoglobin spots on the paper is taken as a rough guide to the amount present, hemoglobin E forms the major component in Mediterranean-hemoglobin E disease and the minor component in the hemoglobin-E trait.

Two additional families in which Mediterranean anemia has occurred have been examined; one of these was included in the report by Minnich *et al.* (*2*) in their study of Mediterranean anemia in Thailand. Only hemoglobins A and F were found, the expected pattern in Cooley's anemia. Further studies of the occurrence and significance of hemoglobin E are currently in progress in Thailand.

References and Notes

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7. Hemoglobin A refers to the normal adult type of hemoglobin; hemoglobin F, to the normal fetal variety; hemoglobin S, to sickle cell hemoglobin; and hemoglobins C and D, to more recently discovered varieties. *Blood* **8**, 386 (1953).
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Experimental Production of Renal Glycosuria, Phosphaturia, and Aminoaciduria by Injection of Maleic Acid*

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Berliner, Kennedy, and Hilton (*1*) reported that the intravenous injection of maleate into acidotic dogs interfered with the renal tubular mechanisms necessary for excretion of acid urine. Impairment of other renal tubular functions also resulted, and the urinary excretion of phosphate was increased. In a footnote the authors commented on a possible reduction of phosphate Tm. We have suggested that one of the effects of vitamin D is to increase renal tubular reabsorption of phosphate (*2*) and became interested in maleic acid as a possible inhibitor of a renal tubule system that is influenced by vitamin D.

When maleic acid, neutralized to *p*H 7.0 with NaOH, was injected intraperitoneally as a 0.1*M* solution into rats fed a low phosphate diet, an increased phosphate loss in the urine occurred. In the following experiments, 6-wk-old rats that had been maintained on a high-calcium, low-phosphorus, rachitogenic ration for 3 wk were placed in metabolism cages permitting quantitative collection of urine uncontaminated with feces or diet. The following substances were determined in the urine by the methods listed: phosphorus, Fiske and Subbarow (*3*); calcium, Kramer and Tisdall (*4*); citrate, Natelson *et al.* (*5*); and