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PUBLICATIONS

Magnitude of the Fetal Hemoglobin Response to Acute Hemolytic Anemia in Baboons is Controlled by Genetic Factors

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ABSTRACT When hemolytic anemia was induced in 26 baboons (*Papio cynocephalus*), aged 7–22 mo, they increased their production of fetal hemoglobin (HbF). Although the resulting reduction in hematocrits and increases of reticulocyte counts were similar in all stressed animals there was marked variability in the maximal rates of HbF synthesis. The maximal levels of HbF attained appeared to fall into three separate groups: low, intermediate, and high. These differences were not related to sex or several measures of erythrocyte metabolism. Animals exposed to repeated episodes of erythropoietic stress after full hematologic recovery demonstrated some variability in their maximal HbF levels attained from one episode to another, but these variations never extended to adjacent classes. The described biochemical and mating data suggest that the magnitude of the HbF response to hemolytic anemia is controlled by genetic factors.

INTRODUCTION

When adult or juvenile baboons were exposed to acute hypobaric hypoxia or when acute hemolytic anemia was induced by phenylhydrazine they increased their production of fetal hemoglobin (HbF)[1] (1, 2). Although this was true for all stressed animals, there was marked individual variability in the maximal rate of HbF synthesis and the maximal level of HbF attained. Animals exposed to repeated episodes of erythropoietic stress, after full hematologic recovery demonstrated much less variability in their maximal HbF levels attained each time. The data, therefore, suggested genetically determined individual variability in the rate of HbF synthesis in response to erythropoietic stress.

Data presented in this paper strongly support the concept of genetically determined variability in HbF response to erythropoietic stress.

METHODS

Acute hemolytic anemia was produced by daily intraperitoneal injections of phenylhydrazine (0.4 ml of a 5% solution/kg) for 5 d (1). All animals received 10 μg of vitamin B_{12} and 0.6 mg of folate on days 4 and 5 of treatment and for 5 d thereafter. HbF levels and hematocrits were determined before, during, and for varying periods after induction of hemolysis (1). Erythrocyte glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, glutathione reductase, reduced glutathione, glutathione peroxidase, and reduced glutathione stability were measured as parameters of erythrocyte antioxidant capacity, inasmuch as phenylhydrazine produces hemolysis by oxidative damage. These studies were kindly performed by Dr. Henri Frischer,

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Received for publication 24 August 1979 and in revised form 17 October 1979.

[1] *Abbreviation used in this paper*: HbF, fetal hemoglobin.

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RESULTS

The maximal HbF levels attained in 26 phenylhydrazine-treated baboons (age at the time of treatment 7–22 mo) are shown in Table I by sex and parentage. They ranged from 1.3 to 59.1%. The histogram of these values of this obviously small sample (Fig. 1) lends itself to the interpretation that there are three phenotypic groups for the HbF response to hemolytic anemia; low (n = 6, range 1–9.9%, mean HbF, 5.2±0.48), intermediate (n = 13, range 10–24.9%, mean HbF, 16.4±0.90), and high (n = 7, range 28–59.9% mean HbF, 40.6±4.04). There does not appear to be any correlation between sex or minimal hematocrit value and phenotypic groups. Reticulocyte counts were obtained on animals 3,433, 3,267 (low phenotypic group), 3,379 and 3,435 (high phenotypic group) during their recovery from hemolytic anemia.

TABLE I
Maximal HbF Levels Attained in 26 Phenylhydrazine-treated Baboons

Father	Mother	Offspring	Sex	Lowest packed cell volume after treatment	Maximal percent HbF offspring
					%
498	2414	3266	Male	22	4.0
498	2410	3267*	Female	20	6.4
498	1588	3941	Male	18	8.0
498	2414	3954	Male	18	14.9
498	1007	3986	Female	20	12.0
498	2234	3975	Male	19	15.9
498	2231	3832	Male	18	16.9
2319	3198	3950	Male	20	15.4
2319	3201	3987	Female	17	34.6
2319	2235	3958	Female	16	30.8
2319	3285	3940	Female	21	14.5
2319	3293	3972	Female	23	19.5
4806	1007	3450	Male	17	17.7
4806	2172	3433*	Female	18	1.3
4806	2234	3508*	Female	21	14.5
426	2843	4009	Male	22	24.1
426	2332	3955	Male	15	18.9
2480	3289	3956	Female	15	39.8
2480	2064	4008	Male	19	51.7
2096	1827	3971	Female	20	4.8
2096	2342	3728	Female	19	16.9
2946	3049	4005	Female	21	59.1
2869	2340	3435*	Female	21	34.8
2479	2064	3379*	Female	20	33.2
2318	2483	3432*	Female	20	12.0
2598	2412	3760	Female	17	6.9

* Animals retreated with phenylhydrazine after full hematologic recovery (Table II). Note that the phenylhydrazine-treated animals were the offspring of 26 separate matings involving 11 males and 23 females.

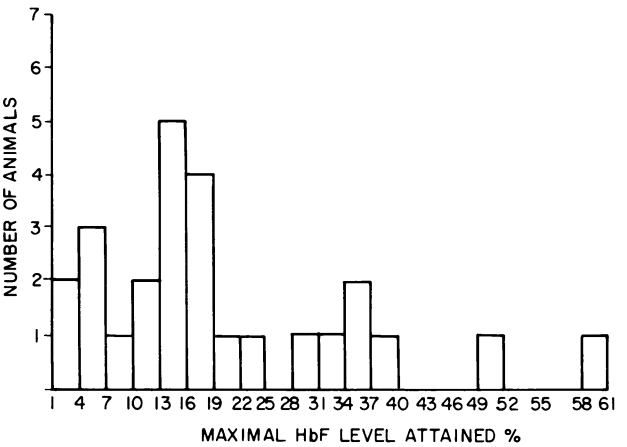

FIGURE 1 Histogram of maximal HbF levels attained in 26 baboons with acute hemolytic anemia produced with phenylhydrazine. These levels fall into three groups: Low: 1–9.9% (mean, 5.2±0.48). Intermediate: 10–24.9% (mean, 16.4±0.90). High: 28–59.9% (mean, 40.6±4.04).

The maximal reticulocyte counts were 71.5, 74.8, 78.1, and 69.3%, respectively, indicating no differences in reticulocyte responses between animals of the low and high phenotypic groups.

Two or three animals from each phenotypic group were exposed to 1–4 additional episodes of hemolytic stress after full hematologic recovery from the previous episode (Table II). The maximal HbF levels attained during the subsequent hemolytic episodes always remained within the same range, and there were no instances of changes in the phenotypic group.

The 26 phenylydrazine-treated animals were the offspring from 26 separate matings involving 11 males and 23 females as shown in Table I. A comparison of the offspring within half-sibships demonstrates that the maximal HbF levels attained always fell into adjacent phenotypic groups. Animal 498 is the father of seven offspring involving six different mates. Three offspring

TABLE II
Maximal HbF Levels Attained in Seven Baboons Exposed to Repeated Episodes of Hemolytic Stress after Full Hematologic Recovery

Animal number	Maximal percent HbF after each episode of hemolytic anemia
	%
3433	1.3, 3.7, 4.0
3760	6.9, 6.0
3267	6.4, 6.8
3508	14.5, 13.1
3432	12.0, 16.8, 10.9
3379	33.2, 46.1, 34.5, 37.4, 34.2
3435	34.8, 27.3

attained maximal HbF levels that fell into the low response group (one-half of all low response animals), and four offspring attained maximal HbF levels characteristic of the intermediate response group. Animal 2319 is the father of five offspring involving five different females. Three offspring fell into the intermediate response group and two into the high response group.

A comparison of the variability within paternal half-sibships to that between half-sibships demonstrated that paternal half-sibs had more similar maximal HbF levels than unrelated individuals ($F = 7.43$, $P < 0.002$), thereby suggesting that the magnitude of the HbF response to hemolytic anemia is under genetic control.

The erythrocyte levels of several enzymes and reduced glutathione (including its stability) were measured to determine whether any of these markers were correlated with the HbF response. Blood samples were obtained from three phenylydrazine-treated animals (one animal from each phenotypic group) 5 wk after the beginning of treatment, and from one untreated animal of similar age. No differences were observed among the treated animals. There was a slight increase of the enzyme levels over the normal control values, probably because of the younger mean erythrocyte age of the treated animals.

DISCUSSION

The available biochemical and mating data suggest that the magnitude of the HbF response to experimentally induced hemolytic anemia is controlled by genetic factors. Even though the mode of inheritance cannot be precisely defined at this time, the presented data support the assumption that the magnitude of the HbF response is controlled at a single locus having two codominant alleles. The observation of three phenotypic groups with 6 individuals in the low, 13 individuals in the intermediate, and 7 individuals in the high response group is suggestive of a binomial distribution. This hypothesis could not be rejected ($\chi^2 = 0.002$, $P > 0.98$), but we realize that it was based on a small sample.

The present study does not exclude other genetic models such as multifactorial inheritance which could also account for the presented data. Because there is considerable intragroup variability, the possibility that minor modifier genes may have an effect on attained maximal HbF levels must also be considered. Additional full sibship data, including data on the magnitude of the HbF response in parents exposed to hemolytic stress would help clarify the genetic basis of the described phenomenon, but at the present time regulations governing our primate facilities do not permit experimental use of animals required for breeding.

In a previous study (3) we showed that bone marrow erythroid cells cultured from animals from all phenotypic groups produced equally high levels of HbF (~90%) and thus the phenotypic differences are obliterated under culture conditions. Therefore, the possibility that the genetic determination is mediated through extracellular factors must be strongly considered.

That the magnitude of the HbF levels and of the number of cells containing HbF is also under genetic control in man has been suggested by recent studies of apparently normal English families (4) and of Saudi Arabian families with sickle cell anemia (5).

ACKNOWLEDGMENTS

This work was supported by National Institutes of Health grant HL 20920-02A1 and by Veterans Administration research funds.

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