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Genetica. — *Postnatal decline of foetal haemoglobin in normals and in haemoglobin* S *heterozygotes.* Nota di GISELA MARTINEZ (*), MARIA ELENA CAÑIZARES (*), LUCIANO TERRENATO (**) e BRUNO COLOMBO (***), presentata (****) dal Socio G. MONTALENTI.

ABSTRACT. — Postnatal decline of HbF was studied in 28 AS subjects and 17 AA controls for six months after birth. HbF disappearance in AS subjects is somewhat delayed as compared to AA babies. A specular pattern is found for HbA behaviour The two phenomena (the delays of both HbF decrease and HbA increase) produce after birth in AS subjects a total Hb pattern not substantially different from that of normal controls.

KEY WORDS: HbF switch; Sickle cell trait; Differentiation.

RIASSUNTO. — *Riduzione dopo la nascita della emoglobina fetale in soggetti portatori di falcemia.* La riduzione dopo la nascita della emoglobina fetale è stata studiata fino a sei mesi di vita in 28 soggetti eterozigoti per l'emoglobina S e 17 soggetti di controllo omozigoti per l'emoglobina A. La scomparsa della emoglobina fetale nei soggetti AS è ritardata rispetto ai soggetti AA, ed un comportamento speculare è osservato per l'emoglobina adulta. I due fenomeni (cioè i ritardi della scomparsa della fetale da un lato e della comparsa della adulta dall'altro) producono nei soggetti AS dopo la nascita un comportamento della emoglobina totale non sostanzialmente dissimile da quello dei soggetti normali di controllo.

INTRODUCTION

During foetal differentiation several haemoglobins are synthesized, the presence of which can be related to the different conditions of loading and unloading of oxygen at various stages of development. The pattern of appearance and disappearance of these haemoglobins is suggestive of a mechanism by which the expression of some genes excludes the activity of other genes [1]. Thus the decrease of ε-chains corresponds to an increase of γ-chains, the synthesis of which is turned off around birth while the synthesis of adult β-chains has been already switched on.

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The switch from foetal (HbF) to adult (HbA) haemoglobin is an example of differentiation associated with a change in the erythrocyte population. It is well known that β-chain synthesis is activated early in foetal life and significant levels are reached at birth. Biosynthesis studies have demonstrated that the very low amount of haemoglobin synthesized in cord blood is accounted for by approximately 50% HbA and 50% HbF [2, 3].

Most of the haemoglobin genetic variants are associated with a reduced rate of synthesis. In the case of common variants the availability of homozygous variant subjects offers the opportunity of verifying the influence of a reduced adult haemoglobin synthesis on foetal-adult haemoglobin switch.

The pattern of foetal haemoglobin (HbF) disappearance after birth has been already compared between normals and haemoglobin S (HbS) homozygous subjects. In the first case the time course of both qualitative and quantitative variations of HbF and adult haemoglobin (HbA) indicates that from birth to approximately 6 weeks only a change in the amount of total haemoglobin takes place, whereas from 6 to 25 weeks there is a change only in composition, HbA substituting HbF which decreases at a rate of approximately 16% of the amount present in the preceding week [4]. In the case of sickle cell disease postnatal decline of HbF has been shown to be slower than normal and moreover, between 6 months and 5 years, correlated with parental HbF levels, thus suggesting a genetic high HbF determinant in *cis* to S gene [5].

In this paper the pattern of HbF disappearance after birth in HbS heterozygotes is presented, with the aim of verifying if in the heterozygous condition HbS gene is able to determine a change in the characteristics of foetal-adult haemoglobin swith.

Materials and methods

28 AS heterozygotes were identified by cellulose acetate electrophoresis [6] among at term newborns delivered during a period of about 6 months at the Obstetric Unit of E. Cabrera Hospital in Havana, Cuba; 17 non-white babies electrophoretically HbA were randomly chosen as controls. Blood was collected at birth and at 15, 30, 45, 60, 90, 120, 150 and 180 days by heel or vein puncture. Total Hb was determined according to *Van Kampen and Zijlstra* [7]; HbF level was determined as previously described by a dilution method which overcame the error introduced by the wide range of HbF values from birth to 6 months [8]. In the 28 cases showing a slow electrophoretic band at pH 8.6, the presence of HbS was confirmed by the identification of the abnormal haemoglobin in one of the parents through electrophoresis and solubility test carried out in a high phosphate buffer [9]. The presence of thalassaemia genes cannot be excluded, but the selection of non-white babies as AA controls should avoid different incidences of these genes in the two samples. Standard statistical techniques were applied to calculate Student t tests between AA and AS samples.

TABLE I.

Postnatal time courses of Hb and Hb F levels (mean ± SE) in AA and AS babies.

Age	AA controls			AS heterozygotes			P (*)
	N	Hb (gr %)	HbF (%)	N	Hb (gr %)	HbF (%)	
Birth	17	20.43 ± 0.49	68.07 ± 2.89	28	19.27 ± 0.35	67.51 ± 1.81	< 0.10
15 days . . .	8	15.50 ± 1.42	59.33 ± 3.78	8	14.50 ± 1.07	69.16 ± 3.17	< 0.10
30 days . . .	7	12.56 ± 0.86	56.07 ± 5.31	22	12.58 ± 0.33	57.07 ± 1.98	ns
45 days . . .	8	11.41 ± 0.46	39.38 ± 5.09	7	11.36 ± 0.34	45.89 ± 5.06	ns
60 days . . .	7	10.19 ± 0.52	34.04 ± 5.40	22	10.64 ± 0.18	43.65 ± 2.22	< 0.10
90 days . . .	4	9.78 ± 0.18	13.10 ± 3.98	22	10.90 ± 0.17	27.37 ± 2.01	< 0.01
120 days . . .	2	10.25 ± 0.65	9.00 ± 4.81	18	10.69 ± 0.19	12.84 ± 2.17	ns
150 days . . .	5	10.70 ± 0.73	3.60 ± 0.86	6	10.97 ± 0.51	6.81 ± 1.28	< 0.10
180 days . . .	4	10.75 ± 0.58	1.80 ± 0.66	14	10.71 ± 0.19	4.14 ± 0.50	< 0.05

(*) For the comparisons b tween the AS babies and the corresponding age-matched AA control babies.

Results and discussion

Table I shows the results obtained for the two measures, Hb gr % and HbF %, while in fig. 1 the same results are reported in terms of total Hb, HbF and HbA gr %.

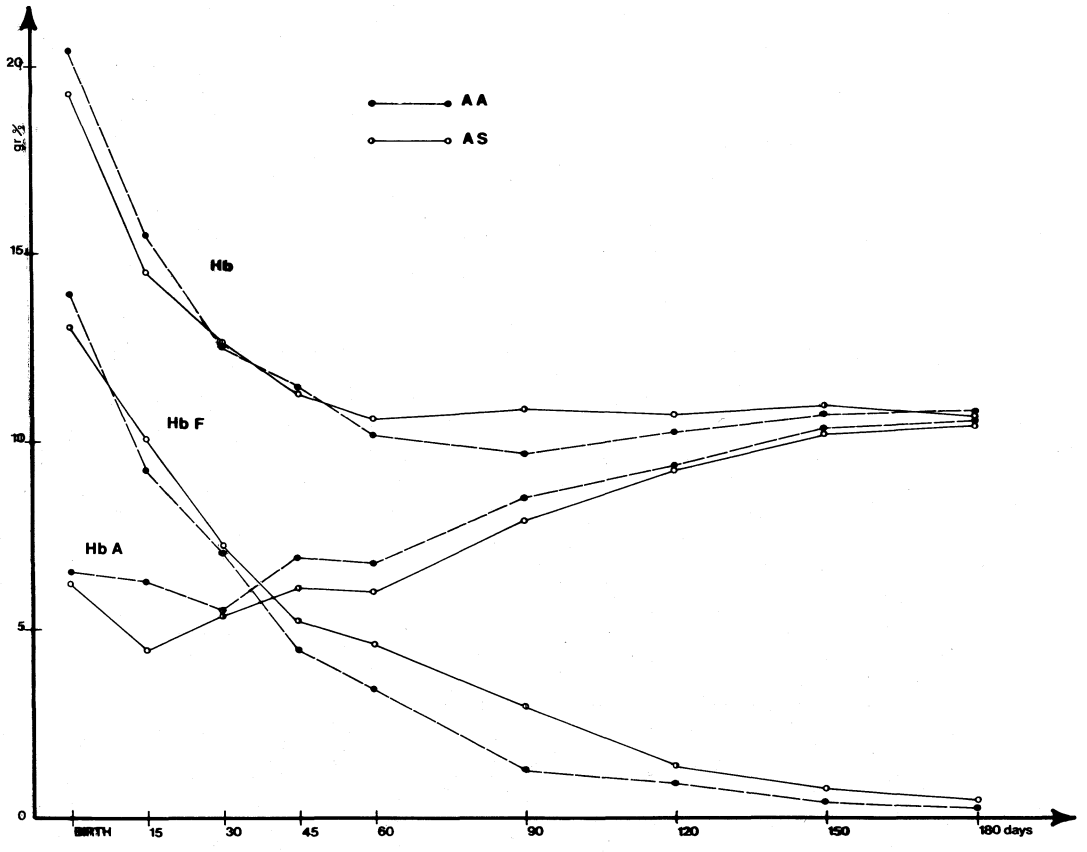

Fig. 1. - Postnatal behaviour of HbA, HbF and total haemoglobin in AA and AS subjects. Sample sizes and standard errors reported in Table I.

It appears clear that the post-natal decline of HbF is somewhat delayed in AS heterozygotes as compared to AA controls; HbF values in AS subjects are higher at all ages, in particular after 2 months, with statistically significant differences at 3 and 6 months, when HbF values are more than double in AS as compared to AA babies. A specular pattern is found for the HbA behaviour; the values are lower in AS than in AA subjects and only after 4 months of life

is no difference appreciable between AA and AS. On the whole, the decline and plateau of total Hb is rather similar in AS and AA individuals.

The results presented here show that after birth HbF behaviour in AS babies is intermediate between AA and SS homozygotes [5]. Available data on the increased values of HbF and F cells in association with S gene (in homo- or heterozygosity) has been interpreted as being due to a factor linked to the S gene [10]. Differential haemolytic selective pressure as been also hypothethesized to explain the delayed HbF decline in SC heterozygotes [11]. On the other hand the reduced levels of non–foetal haemoglobin in AS subjects is more easily explained by the reduced activity of the S gene, still not compensated by the hyperactivity of the residual A gene: in fact in AS newborns the share of HbS with respect to other haemoglobins is higher than in adults [12].

Under these circumstances it may be considered not obvious that two apparently independent phenomena (the delays of both HbF decrease and HbA increase) produce after birth in AS subjects an overall Hb pattern not substantially different from that of normal controls.

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