Postnatal Fetal and Adult Hemoglobin Synthesis in D1 Trisomy Syndrome

By Harry Bard

Studies were carried out during the neonatal period in three infants with D1 trisomy syndrome to measure the proportion of fetal and adult hemoglobin being synthesized. These values were compared on the one hand to those previously reported from samples obtained from cord blood of normal newborn infants ranging from 25 to 43 wk gestation, and on the other hand to those values determined in critically ill infants of the same postconceptional age. Blood samples were incubated in an amino acid mixture containing 14C-leucine followed by column chromatography on DEAE Sephadex for separation of fetal and adult hemoglobin fractions.

Liquid scintillation counting was carried out on the hemoglobin fractions. In infants with the D1 trisomy, the delay in transition to adult hemoglobin synthesis was 7–8 wk behind that expected for their postconceptional ages, and there was no accelerated transition to adult hemoglobin synthesis in the one case studied beyond the early neonatal period. Unlike the D1 trisomy infants, the critically ill controls showed no retardation in their transition toward adult hemoglobin synthesis. The duplication of the genes in one of the 13–15 chromosome groups is a factor that delays the developmental synchrony of hemoglobin synthesis.

The reciprocal relationship between fetal (F) and adult (A) hemoglobin during pre- and postnatal life provides an excellent opportunity to study the mechanisms of protein synthesis. Little is known about the factors that regulate F and A hemoglobin synthesis during the perinatal period. The breakdown of this control in various disease states also remains unclear.

There are several clinical syndromes associated with chromosomal anomalies in which the orderly sequence is disturbed. In the trisomy D1 syndrome, F hemoglobin exists in higher concentrations than in normals at birth, while A hemoglobin has been discovered to be in excess in newborns with trisomy G (Down’s syndrome) and in one reported case of C/D translocation.

The purpose of this study was to measure the relative rates of A and F hemoglobin synthesis and thereby to estimate the delay in appearance of A hemoglobin and the disappearance of F hemoglobin in newborns with the D1 trisomy syndrome, as compared to normal newborn infants, and also to deter-
mine if there would be a rapid switchover to A hemoglobin synthesis beyond the early neonatal period. Since all three trisomy D1 infants expired before the age of 1 mo, a group of critically ill infants of similar postconceptional age were also included in the study as controls.

The proportional synthesis of F and A hemoglobin can be determined by measuring the incorporation of $^{14}$C-leucine into the alpha, beta, and gamma polypeptide chains formed during the in vitro incubation of reticulocytes. Thus, hemoglobin synthesis can be measured in newborn infants with D1 trisomy syndrome and compared with normal newborn infants appropriate in weight for gestational age (A.G.A.).

MATERIALS AND METHODS

Three term, newborn infants with the typical trisomy 13–15 (D1 trisomy) phenotype and all having cyanotic heart disease and their genotype confirmed by chromosomal analysis at the University of Colorado Medical Center were used for this study. One infant was born at the Center; the other two were referred at the age of 6 days and 3 wk, respectively. The infant born at the Center was sampled twice at 2-wk intervals, and the others were sampled once on admission. All three expired before the age of 1 mo. For controls, the relative amount of fetal hemoglobin to total hemoglobin synthesis was also determined in three critically ill infants having congenital cyanotic heart disease. The first with a ventricular septal defect was studied at 2 wk of age, the second with a hypoplastic left heart at 3 wk of age, and the third with a ventricular septal defect was studied after having undergone surgery for an intestinal obstruction.

The relative proportions of F and A synthesis were determined by methods described in detail previously. In brief, heparinized blood samples were obtained and incubated under sterile conditions. To 2.64 cc of an incubation mixture prepared by a modification of the procedure devised by Boorsook et al., 1 cc of packed (reticulocyte-enriched) red cells, 0.15 ml of ferrous ammonium sulfate (10.5 mg in 10 ml of 0.9% NaCl), and 0.1 ml $^{14}$C-leucine (DL-leucine-1-$^{14}$C, obtained from Amersham Searle, specific activity 55.2 mCi/mole) were added.

After a 6-hr incubation carried out with agitation under air at 37°C the cells were washed and lysed, and the hemolysate was desalted and purified by passage through a Sephadex G-25 column. The purified hemoglobin solution containing 60–80 mg hemoglobin was then subjected to column chromatography on a DEAE Sephadex A-50 medium (Pharmacia Fine Chemicals) using columns 45 cm × 2.5 cm; the Sephadex height in the columns at the start of the elution varied between 18 and 25 cm. The hemoglobins were eluted at room temperature (20–21°C) using a decreasing pH gradient (7.9–7.1) of 0.5 M Tris-HCl at a flow rate of 20–26 ml/hr. Fractions of 4 ml were collected. The absorbency of the eluate was continuously measured at 280 nm.

Liquid scintillation counting of the adult and fetal hemoglobin fractions was carried out after preparing the samples in the following manner. To 0.5 cc of the eluted fraction, 1.1 cc of Bio-Solv-BBS-3 (Beckman) as a solvent and 0.1 cc 50% hydrogen peroxide as a decolorizing agent were added. The vials were then placed in a water bath at 50°C for 30 min. Finally, 12 cc of “cocktail” containing 4 g of preweighed Omnifluor (New England Nuclear) in 1000 cc toluene were added to the vials. Counting was then performed in a unilux 11 A liquid scintillation system (Nuclear-Chicago). Counting efficiency varied between 77%–81% on an external standardization quench curve. The external standard counts were very similar from one sample to another; they varied from 2.1% to 1.7% from the mean.

To determine whether there were any differences in quenching between A and F hemoglobin, a known standard activity was added to samples of different optical densities of either adult or fetal hemoglobin. The observed count rates of A and F hemoglobin were very similar; the mean for A hemoglobin was 320,512 cpm, SD 10,324, and the
D_{1} TRISOMY SYNDROME

mean for F hemoglobin was 331,401 cpm, SD 11,010.

The method used in calculating the ratio of fetal hemoglobin synthesis to total hemoglobin synthesis was to plot the counts per minute vs. hemoglobin fractions on uniformly thick graph paper (Keuffel & Esser, No. 10 5663), then to cut out carefully the areas under each of the hemoglobin peaks and to weigh them without handling on a semimicro Mettler \textit{H} 20 balance, which provides a nonambiguous reading to 0.01 mg.

RESULTS

Figure 1 represents the chromatograms and the counts per minute of hemoglobin fractions from the three D_{1} trisomy infants studied. The adult (A) and
fetal (F and F1) hemoglobin peaks were symmetrical in shape, and the separations were complete. The proportion of fetal to total hemoglobin being synthesized decreased with increasing postnatal age.

In Fig. 2, the amount of radioactive hemoglobins in the three cases studied were compared with the calculated regression line of fetal hemoglobin synthesis determined in cord blood from newborn A.G.A. infants. All three were
synthesizing excessive amounts of fetal hemoglobin for their postconceptional ages. The amounts of radioactive fetal hemoglobins in the D1 trisomy infants corresponded to levels found in A.G.A. newborn infants 7–8 wk less mature in postconceptional age than the D1 trisomy infants. The same delay in the appearance of A hemoglobin synthesis was observed in the one case studied twice during a 2-wk interval. The amounts of fetal hemoglobin being synthesized in the three critically ill infants were on or just below the 95% confidence limit of the A.G.A. newborn infants 42–43 wk in gestational age.

DISCUSSION

In this study, the delay of the appearance and disappearance of A and F hemoglobin synthesis in the 13–15 trisomy infants lagged 7–8 wk behind the preterm and term infants previously reported. In the one case sampled twice at a 2-wk interval, the rate of transition, although delayed, was parallel to the normal regression line.

The delay in switchover in the D1 trisomy infants could not be related to their "sick" condition, since in the three critically ill infants there was no retardation in their transition from fetal to adult hemoglobin synthesis.

These findings support the view that there are genetic factors involved in the control of the developmental conversion from fetal to adult hemoglobin synthesis and that 13–15 trisomy causes a delay in this biological time table. The possibility that some of the loci controlling the sequence in the transition from gamma to beta polypeptide synthesis are located on the chromosomes triplicated must be entertained. With the new staining techniques now available, the exact chromosomes implicated in the 13–15 group should be easily detected.

REFERENCES

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