BRIEF REPORT

Fetal Hematopoiesis—Case Report

By Miriam Rosenberg

ALTHOUGH OBSERVATIONS of human embryonic and fetal hematopoiesis have been reported, most specimens studied early in gestation have been more or less macerated on account of the cause of death itself or the period spent in utero between death and abortion. Recently a living 11 week, 4.7 cm. fetus was delivered during a therapeutic abortion by Caesarian section at Yale-New Haven Hospital; it died of asphyxia within eight minutes and fresh, actively mitotic hemopoetic tissue was obtained for study.

Several mammalian proteins have been reported to exist in distinctive molecular forms during the fetal period; the possibility must be considered that these represent artifactual abnormalities due to tissue deterioration and are not normally present in the living fetus. For this reason, an electrophoretic survey of enzymes in fresh tissue homogenates of this sound fetus was also undertaken.

MATERIALS AND METHODS

The mother was Grav. IV, Para. III, thirty-eight years old, white, of Anglo-Saxon-German ancestry, in good general health but under psychiatric treatment for psychoneurotic anxiety state, which was the indication for abortion; she has taken Chloriazepoide (Librium), 50 mg./day, for two years and has not undergone ECT. Caesarian delivery had been initiated with the first pregnancy on account of fetopelvic disproportion and the present operation proceeded without complication. The remainder of her history and clinical examination offered no indication to anticipate abnormalities in the fetus; autopsy of the fetus revealed no sign of disease other than atelectasis and gross prematurity.

The fetus was dissected with sterile precautions, and .2 mm. sections of spleen, liver, yolk sac, and femur were transferred aseptically and rapidly to tissue culture observation chambers. These were incubated at 37°C under air and observed by phase microscopy for eighteen hours or until dedifferentiation and loss of architecture appeared; all observations were begun within 25 minutes of death. Samples of these tissues as well as of kidney, thymus, thyroid, pancreas, omentum, sternum, skull, and humerus were also preserved in Bouin’s and Zenker’s fluids, sectioned and stained with H. & E. or Giemsa. Microscopy and photography were performed with a Carl Zeiss attachment camera on microscope standard WL.

For tissue-specific isozyme surveys, individual organs were dissected, washed of excess blood in isotonic saline, homogenized in equal volumes of .1M phosphate buffer, pH 7, at 4°C, and centrifuged at 17,000g., 4°C, 20 minutes. The supernate from each tissue was applied within three hours to starch or disc acrylamide gels, and electrophoresis was performed by standard methods. At the end of the runs gels were incubated in specific stains for glucose-6-PO₄-dehydrogenase, galactose dehydrogenase, 6-phosphogluconate dehydro-
Fig. 1.—(A) Culture of fetal liver, 30 min. postmortem. Phase, X 640. A site of active intravascular erythrocytosis: peninsulas of reticular parenchyma extend into a large sinusoid in which three mitoses, (arrows) of cells at three different stages of maturation, proceed. Mitoses could be followed from beginning to end. Such cells could be followed in culture from prophase through telophase usually about twenty minutes; there averaged five mitotic figures per three hundred hemopoietic cells.

Fetal and adult hemoglobin concentrations were determined by alkali denaturation and by spectrophotometry on eluates from starch gels after electrophoresis; results from the two determinations were averaged. Oxygen-carrying capacities were measured by standard methods. Two macerated fetuses, products of therapeutic abortion by saline injection, one 9 and the other 12 weeks' gestation, were used to repeat certain findings. Both had remained in utero at least 20 hours after saline injection.
Fig. 1.—(B) Liver section, stained with giemsa. Zeiss planapo Lens, x 256. Some basophilic erythroblasts and proerythroblasts (arrows), identified by morphology in giemsa, are seen attached to the parenchyma but otherwise erythropoiesis proceeds within the sinusoids. Tissue fixed in Bouin’s immediately postmortem.

Observations

The liver (Fig. 1) was actively erythropoietic. The centrolobular architecture of the adult was loosely sketched out by a meshwork of reticuloendothelial-type parenchyma, surrounding sinusoids into which maturing erythroid cells spilled. Pro- and basophilic erythroblasts, [identified by morphology in Giemsa] were included in the reticuloendothelial substratum but all subsequent stages of erythroid maturation floated free and divided in the
Fig. 2.—Liver imprint, prepared immediately postmortem, stained with Giemsa. Zeiss planapo lens, x 2000. Erythroblastic island seen in hepatic sinusoid. All stages of maturation surround an RE cell.

sinusoids, as did entire erythroblastic islands—RE cells surrounded by a halo of maturing erythroid cells (Fig. 2)—identical to those seen in adult marrow. Despite the predominance of an intravascular site for erythropoiesis, only 1 per cent of peripheral blood cells were nucleated (Fig. 3). There was
Fig. 3.—Peripheral blood cultured in autologous plasma for ten minutes post-mortem. Phase, x 1600. Only 1 per cent of the cells are nucleated despite intravascular site of hepatic erythropoiesis.

no recognizable myelopoiesis in the liver but there were megakaryocytes (Fig. 4) comparable to those in adult marrow.

The spleen (Fig. 5) exhibited no evidence of hematopoiesis or lymphopoiesis and appeared to serve only as a graveyard for large erythroblasts which were seen sequestered in splenic sinusoids at various stages of degeneration. Splenic RE cells could be seen actively engulfing deteriorated erythrocytes in tissue culture. The sequestered erythrocytes resembled the primitive erythroblast of mice, with checkerboard nuclei, dense eosinophilia in Giemsa, and blastlike size.

The shafts of femur and humerus were sparsely myelopoietic but not erythropoietic (Fig. 6), and the tissue was otherwise fibroblastic.

The yolk sac was completely fibrotic and there was no indication of hematopoiesis in any other tissue examined.

**Enzyme Electrophoresis**

By criteria of starch gel electrophoresis, alkali denaturation, and oxygen dissociation curve, 37 per cent of the hemoglobin in liver homogenate was adult type Hb.A (Fig. 7); nevertheless the peripheral blood and all other organs contained less than 4 per cent Hb.A, the remainder being Hb.F. These findings were validated by using the other two fetuses, whose livers, though
macerated, contained 22 per cent (9 week fetus) and 30 per cent (12 week fetus) of the Hb.A-like pigment (these values cannot be considered precise since the livers were exceedingly soft and could not be rinsed of excess blood).

Three forms of G6PD were seen (Fig. 8), equally spaced and of equal intensity; all three were observed in liver, kidney, and skeletal and cardiac muscle homogenates while only the anodal two were observed in blood, brain, skin, lung, GI organs and spleen. The cathodal form was exceedingly
labile, disappearing at 4 C. within 48 hours of tissue homogenation; the anodal forms lost all activity more slowly but were undetectable in all tissues within 96 hours of homogenization. No G6PD activity was detectable in the tissues of the two macerated fetuses, probably due to instability of the enzyme. On account of reports of a cathodal H6PD, reacting with galactose-6-PO$_4$ in human liver, the same gels were stained with galactose-6-PO$_4$ as substrate; a single fourth more cathodal band from liver, and no other organ, appeared, but this was cathodal to the third form of G6PD seen in this fetus. Once recognized, it was seen to stain faintly with glucose-6-PO$_4$ as well.

All other enzymes examined (page 4) exhibited the electrophoretic mobility typical of adult tissues, except hexokinase, whose pattern resembled its fetal variety previously reported.\(^{26}\)
Fig. 6.—Femoral cavity imprint, prepared immediately postmortem, stained with Giemsa. Zeiss planapo x 1000. Islands of granulocytic myelopoiesis are seen scattered loosely in a fibroblastic matrix.

**DISCUSSION**

Points upon which this study introduces new or contradictory information include:
Fig. 7.—Starch gel electrophoresis, pH 8.6, of fetal liver homogenate, adult hemolysate and fetal hemolysate. Benzidine stain. Fetal liver contains 37 per cent Hb.A-like pigment even though peripheral blood contains only 8 per cent. The appearance of greater intensity in the Hb.A band is misleading and due to diffusion of the intense stain.

1) Intravascular hematopoiesis is almost never seen in the adult, even during ascendance of extramedullary centers.15
2) The rarity of nucleated erythrocytes peripherally despite the predomi-
Fig. 8.—Disc acrylamide electrophoresis pH 9.1, 7 per cent gel. Glucose 6-PO₄ dehydrogenase stain. Three equally-spaced bands of equal intensity are seen. There is a faint cathodal doublet band in gel #1, the liver, which appears to represent H 6PD. The anodal band migrated as adult G6PD B. Organs represented include: (1) liver; (2) skeletal muscle.

Nance of erythropoiesis in hepatic sinusoids suggests a cellular barrier which prevents access of immature cells to extrasinusoidal circulation.

3) Sequestration and destruction of erythrocytes by the spleen has not been described, and contradicts a report of hematopoiesis in the spleen at this stage. One does not of course know, without labeling cells, that this is so but the disintegrated appearance of erythrocytes in splenic sinusoids, and their active engulfment by splenic RE cells (Fig. 5), are quite suggestive. The morphologic similarity of the sequestered cells to primitive type erythroblasts of mice is striking; it appears that such a primitive clone is present in humans as well. Possibly all attain senescence at the same time, or else at this stage of development the presence of primitive erythrocytes is disadvantageous so that a mechanism has evolved to destroy all at once. Inasmuch as section and imprint of the spleen revealed the same appearance, it is probably not a postmortem phenomenon.

4) Restriction of myelopoiesis to the marrow cavities suggests refutation of the pluripotent stem cell theory, but it is more likely that organ-specific microenvironments which induce one cell line or the other can change drastically during development.

5) The extraordinarily high level of HbA located exclusively in the fetal liver has not been described. Possibly the liver cells themselves synthesize
Hb.A not intended for erythrocytes; essentially every cell of an organism carries the entire genome,\textsuperscript{17} and the oxidase activity of Hb.A might be useful to liver at this stage in development. In a similar manner, Hb.F, which declines late in gestation, occasionally reappears during adulthood in severe hematologic stress like anemia;\textsuperscript{18} presumably this is an adaptive mechanism. Alternatively this may be a benzidine-positive, red protein whose fetal form might behave electrophoretically, spectrophotometrically, and in O\textsubscript{2} carriage, as adult Hb.A; the coincidence seems improbable but is currently under investigation with immunochemical methods.

Another possibility is that Hb.A-containing erythrocytes are sequestered in the liver until a later stage. It is difficult to imagine a function for such a mechanism, particularly since the erythrocyte life span is only four months, even in the fetus.\textsuperscript{19}

6) Unfortunately it was not possible to maintain G6PD activity in fetal tissues sufficiently long to study the origin of the three electrophoretic forms. Using adult blood as a marker, the most anodal fetal G6PD was parallel to normal adult type B G6PD.\textsuperscript{20} Certain experimental conditions can split the single anodal adult G6PD into two bands;\textsuperscript{20} however, these are more closely spaced than the two anodal fetal bands seen here and are not of equal intensity. A cathodal G6PD has been described in human liver which reacts with galactose-6-PO\textsubscript{4} as well; this form did appear faintly in the fetal liver and was not the same as the third, cathodal G6PD also seen in fetal liver.

Three equally-spaced bands of equal intensity suggest that the cathodal band represents an allelic mutation and the middle band, a hybrid heteropolymer. Quite possibly, the cathodal band, like fetal hemoglobin, represents expression of a gene most active in fetal life, while the center band represents another such gene or a hybrid product between the adult and fetal G6PD gene products. Although human G6PD is probably a hexamer,\textsuperscript{21} so that theoretically random recombination of two different subunits should yield at least 100 hybrid products, restriction of hybridization can yield any final number.\textsuperscript{22} It comes as no surprise that distinctive fetal forms of G6PD exist since such forms have been described for many proteins; however, it is chancy to base any conclusions on findings in a single case.

\textbf{SUMMARY}

An unusually well-preserved eleven-week, 4.7 cm. human fetus exhibited active erythropoiesis in liver, and myelopoiesis in femur and humerus; the spleen was the site for sequestration and destruction of primitive erythrocytes, and the yolk sac was fibrotic. High proportions of adult type hemoglobin (A) were found in liver homogenate, and an electrophoretically distinctive fetal form of G6PD was observed.

\textbf{SUMMARIO IN INTERLINGUA}

Un inusualmente ben preservate feto human de un etate gestatori de dece-un septimanas e un longor de 4,7 cm exhibiva active erythropoiese in le hepate e myelopoiese in femore e humero. Le spleen esseva le sito de sequestration e destruction de erythroeytos primitive,
FETAL HEMATOPOIESIS

...le sacco vitellino eserci fibrotico. Alte proportions de hemoglobina de typo adulto, i.e., hemoglobina A, eserci trovate in homogenato hepatic, e un electrophoreticamente distincte forma fetal de dehydrogenase de 6-phosphato de glucosa eserci observe.

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