Durable Normal Erythropoiesis in Organotypic Cultures of Guinea Pig Foetal Liver

By Germano Salvatorelli, Anna Maria Gulinati and Paolo Del Grande

During the embryonic and fetal development of mammals, erythropoiesis, first localized in the yolk sac, continues in some transitional blood forming organs, such as liver and spleen; only in the second half of the fetal period does erythropoiesis become definitely localized in the bone marrow.

The differentiation of erythroid elements is incompletely understood, although extensive work has demonstrated that erythropoiesis is under the control of a hormone (erythropoietin) which acts on the primitive stem-cell precursor of the erythroblast. Anoxic and anemic conditions greatly increase the production and consequently the circulatory level of erythropoietin, and routine methods are available for its quantitative estimation in vivo.

Culture of erythropoietic tissue cells in vitro is widely employed for several purposes; but most experiments have been carried out with short-term cultures. Anemic plasma1 or purified erythropoietin2 show a favorable action in maintaining or reactivating3 the hepatic erythropoiesis of the mouse fetus in vitro.

In experiments with histiotypic cultures,2 the duration and maintenance of erythropoiesis in vitro did not exceed 4 hours. Gallien-Lartigue3,4 cultivated explants of fetal liver for about 10 days utilizing the organotypic technic culture of Wolff and Haffen; but it should be noted that the differentiation of stem-cells into erythroblasts stopped after the fifth day whereas the maturation of erythroblasts into erythrocytes continued a little longer.

It was concluded that the stem-cells capable of reacting to exogenous administration of erythropoietin disappear either in consequence of their differentiation into erythroblasts, or that aging and death of the cells occurs in these experimental conditions, presumably due to the lack, in the culture media, of nutritional and hormonal factors necessary for the maintenance and renewal of this particular class of cells.

Working with hematopoietic organs of the chick embryo and utilizing the culture method of Wolff and Haffen,4 it has been possible to maintain myeloid5 and spleen6 erythropoiesis in long-term cultures (12–15 days) under the action of erythropoietin-like factors from the embryonic liver.

Therefore it seemed interesting to test the action of anemic serum on the erythropoietic fetal liver of another species of mammal (guinea pig), with the object of prolonging normal erythropoiesis in vitro; we believe that a normal
DURABLE NORMAL ERYTHROPOIESIS

Table 1.—Liver Hemopoietic Tissue Differentials (per cent) In Vivo and Cultured on Normal and Anemic Serum

<table>
<thead>
<tr>
<th></th>
<th>Normal Fetal Liver in vivo</th>
<th>Fetal Liver Cultured on Normal Serum for 4 Days</th>
<th>Fetal Liver Cultured on Anemic Serum for 14 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemocytoblasts</td>
<td>0.4</td>
<td>—</td>
<td>3.6</td>
</tr>
<tr>
<td>Pro erythroblasts</td>
<td>1.2</td>
<td>—</td>
<td>6.8</td>
</tr>
<tr>
<td>Basophilic erythroblasts</td>
<td>5.7</td>
<td>—</td>
<td>8.4</td>
</tr>
<tr>
<td>Polychromatophilic erythroblasts</td>
<td>10.8</td>
<td>0.6</td>
<td>12.6</td>
</tr>
<tr>
<td>Orthochromatryn erythroblasts</td>
<td>2.9</td>
<td>8.0</td>
<td>21.4</td>
</tr>
<tr>
<td>Erythrocytes</td>
<td>78.3</td>
<td>89.0</td>
<td>46.0</td>
</tr>
<tr>
<td>Mitosis</td>
<td>0.2</td>
<td>—</td>
<td>0.2</td>
</tr>
<tr>
<td>Leukoblasts and leukocytes</td>
<td>0.5</td>
<td>2.4</td>
<td>1.0</td>
</tr>
</tbody>
</table>

erythropoiesis in vitro could be helpful as a standard model for several problems.

METHODS

Explants of guinea pig fetal liver (30-40 days gestation) were cultured by the technic of Wolff and Haffen on fragments of vitelline membrane of nonincubated hen egg7 placed on 1 ml. of a semisolid agar-agar gel medium. Four to six pieces of liver about 0.5 cu.mm. were placed on the same vitelline membrane in each “watch glass” and then incubated at 38 C. In all, about eight hundred explants were cultured by this technic.

The composition of the basic culture medium was as follows:

- gelose 1 per cent in Gey’s liquid 56 per cent
- chick embryo extract (8 3 days inc.) 22 per cent
- anemic guinea pig serum 22 per cent

To prepare the anemic serum, adult guinea pigs were injected subcutaneously with a buffered solution of phenylhydrazine (40-60 mg/Kg.) for 2 days, and serum obtained from blood taken by heart puncture after 2 days, i.e., at the fourth day after the beginning of the experiments.

In control cultures the liver explants were used on the above indicated medium, containing normal guinea pig or horse serum instead of anemic serum.

Morphologic observations were made in May-Grunwald-Giemsa stained smears and imprint of the liver explants. By this technic blood cells are well spread allowing a clear differential cell count (1000 cells examined for selected specimens).

The relative numbers of different blood cells have been expressed as percentages.

RESULTS

**Fetal Guinea Pig Liver Cultured on Media Containing Normal Guinea Pig or Horse Serum**

The culture medium of Wolff and Haffen,4 enriched by normal guinea pig or horse blood serum does not maintain erythropoiesis in organotypic cultures of fetal guinea pig liver. Even after four days in culture the immature elements of the erythroid series are (see Table 1) extremely rare. The proerythroblasts and the basophilic erythroblasts disappear, and the percentage of polychromatophilic erythroblasts is very low.

In contrast normoblasts are present in good numbers and this would seem to indicate that the maturation process of erythroblasts into erythrocytes continues, whereas the differentiation of stem-cells into erythroblasts stops; nevertheless degeneration does occur in normoblasts and erythrocytes. The disappearance of the stem-cells from the fourth day of culture seems to indicate that the culture conditions are incompatible with the needs of these cells.
Figs. 1 and 2.—Smears of guinea pig fetal liver cultured for 14 days on anemic serum: proerythroblasts, basophilic, polychromatophilic (one of which is in mitosis; Fig. 2) and orthochromatic erythroblasts are present.

Fetal Guinea Pig Liver Cultured on Medium Containing Anemic Guinea Pig Serum

On the contrary the serum of anemic guinea pig is capable of maintaining erythropoiesis in long-term cultures (see Table 1 and Figs. 1 and 2).

A comparison of the cellular composition of the explants cultivated on anemic serum against that of the liver in vivo demonstrates that there are no appreciable differences, contrary to what was observed in explants on normal serum. During the whole period erythropoiesis continued normally, and after 14 days the percentage of stem cells, proerythroblasts, basophilic and polychromatophilic erythroblasts is in fact higher that at the time of explantation. Mitoses occur as in controls, and the erythrocytes do not have the degenerate appearance seen in cultures on normal serum.

DISCUSSION

Present experiments demonstrate that anemic guinea pig serum is capable of maintaining the erythropoiesis of organotypic cultures of fetal guinea pig liver for at least 14 days. This result seems interesting, for, as far as known by us, other experiments have been less successful. Using the same technic, Gallien Lartigue\textsuperscript{1,3} remarked that, in organotypic cultures of fetal mouse liver, the erythropoietic differentiation slowed after 5 days and stopped definitely after 7 days.

Presumably the improved results gained in present experiments are due to the use of fragments of vitelline membrane; in fact, as demonstrated by Wolff,\textsuperscript{7} explants cultured on solid media have a tendency to contract into a spheroidal mass, and as a consequence the inner layers degenerate because of insufficient nutrition and respiration.

On the contrary, the vitelline membrane allows the explants to remain
spread as a thin layer with a greater surface, and the inner cells find optimal conditions for their vital functions.

The action of the anemic serum on the maintenance of erythropoiesis in vitro seems to be due to the stimulation of the differentiation of stem cells into erythroblasts with possible influence on the more distal components of the nucleated erythroid cell series. In the absence of erythropoietin, cytodifferentiation stops during the first days of culture, whereas the maturation of normoblasts is able to continue for a few days. In addition the number of stem-cells diminishes considerably and after the fourth culture day they disappear. In cultures on anemic serum their number is unaltered even after two weeks of culture; this seems to indicate that it displays a favorable action in the maintenance of the pool of undifferentiated cells in the explants.

**SUMMARY**

It is demonstrated that anemic guinea pig serum maintains erythropoiesis in organotypic cultures of fetal guinea pig liver for at least 14 days. The action of the anemic serum seems to be due to the stimulation of the differentiation of stem-cells into erythroblasts. If fetal guinea pig liver is cultured on normal serum the cytodifferentiation stops during the first days of culture whereas the maturation of normoblasts can continue for a few days. The number of stem-cells diminishes considerably and after 4 days they all disappear.

On the contrary in culture on anemic serum, their number is unaltered even after two weeks in culture and this seems to indicate that the anemic serum displays a favorable action in the maintenance of the pool of undifferentiated cells in the explants.

**SUMMARIO IN INTERLINGUA**

Es demonstrate que sero de porcos de India anemic mantene Ic erythropoiese in culturas organotypic de hepate fetal de porcos de India durante al minus 14 dies. Le action del sero anemic pare esser le effecto de un stimulation del differentiation de cellulas primordial ad in erythroblastos. Quando hepate fetal de porcos de India es culture in sero normal, le cytodifferentiation se arresta durante le prime dies del cultura, durante que le maturation de normoblastos pote continuar durante plure dies. Le numero del cellulas primordial declina considerabilemente, e post 4 dies illos omnes disparare.

Del altere latere, in culturas con sero anemic, lor numeros non es alterate mesmo post duo septimanas in le cultura, e isto pare indicar que le sero anemic exerce un effecto favorable super le mantenientia del pool de nondifferentiate cellulas in le specimen explantate.

**REFERENCES**

5. Salvatorelli, G.: L'influence favorable


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