15

22

New Istanbul Contribution
to Clinical Science 12, 102-109 (1977)

Congr. Intern. Soc. Haematol.,
Europ. and Afric. Div., Istanbul (1977)

A NEW CONCEPT OF HAEMATOPOIETIC STEM CELL DIFFERENTIATION IN THE YOUNG MAMMALIAN ORGANISM

VALET, G. , HANSER, G. and RUHENSTROTH-BAUER, G.

A NEW CONCEPT OF HAEMATOPOIETIC STEM CELL DIFFERENTIATION IN THE YOUNG MAMMALIAN ORGANISM

VALET, G.*, HANSER, G. and RUHENSTROTH-BAUER, G.

Max-Planck-Institut Institut für Biochemie, Abtellung für experimentelle Medizin, D-8033 Martiusried bei München

The haematopoietic development of the mammalian organism is not terminated at birth. In the erythropoietic system several cellular parameters change: the foetal haemoglobin is gradually replaced by adult haemoglobin (4) and furthermore depending on the particular species, membrane antigens (10), enzyme concentrations (8) and other cellular constituents (8) may alter. In the leukopoietic system full immunocompetence is not reached until some time after birth (9).

An important question to be answered is how the changes in the hematopoietic system are accomplished by the organism. Two basic mechanisms are possible. Cell parameters may change either a) because the cells of a given cell line are produced from day to day with slightly altered parameters or b) because the organism first produces cells with one set of cellular parameters and then, from a certain time on, new cells with a different set of parameters. The cells of the first population are eliminated by the organism with time. Until now it has been difficult to distinguish experimentally between the two possiblities. In the following we show that in mammalian organisms, studied by us, the erythropoietic development after birth is accomplished by means of the second mechanism. This conclusion is mainly based on experimental data obtained from fast flow cytometers. Flow cytometers allow the measurement of volume, fluorescence or light scatter signals from cells at rates of 1000-2000 cells/sec (5). Large and representative samples can be measured in a short time, comparatively few cells are needed and the data can be

efficiently processed by computers. The measurements reported here were made with the electrical cell sizing instrument Metricell (7) which has recently been developed in our department. The volume distribution curves of the erythrocytes were analysed with a special computer program (13).

The starting point of our experiments was the observation that the volume distributions curves of the circulating erythrocytes of several mammals change significantly during postnatal development (17, 15, 13, 12). The erythrocytes of the rat, for example, are greater immediately after birth than in the adult animal. Furthermore the volume distribution curves of the erythrocytes during the first two months of life show two and sometimes three volume peaks. This indicates the presence of several erythrocyte populations each with different mean volume. A total of *five* populations of erythrocytes are observed in the rat during the first three months of life (Fig. 1a), the fifth population is the definitive one during adult life. An additional erythrocyte population M with a large volume can be induced in adult animals during precipitated erythropoiesis after whole body X-irradiation (16) or after bleeding (11). The large erythrocyte population M disappears gradually from the circulation. Until its disappearance, it remains a separate entity which does not merge with the normally sized erythrocyte population.

The number of erythrocyte populations varies in different mammalian species: *three* populations were found in the mouse, rabbit (Fig. 1b), sheep (Fig. 1c) and goat and only *two* in the guinea pig (Fig. 1d).

It is important to realise that the erythrocyte populations vary in a number of parameters besides that of volume. Depending on the animal species these can be the electrophoretic mobility (12, 14), the antigen pattern on the cell membrane, the sodium and potassium pumps of the cell membrane, the intracellular Na/K concentration (12) and the haemoglobin type. For example in the sheep haemoglobin F (Fig. 2) is located in erythrocyte population I (Fig. 1c) and haemoglobin AB (Fig. 2) in population II and III (Fig. 1c). In addition it is likely that haemoglobin C, if present, is located in the population II erythrocytes (12). The different erythrocyte populations in sheep do not transform into each other by volume changes (12). The location of different types of haemoglobin in specific erythrocyte popula-

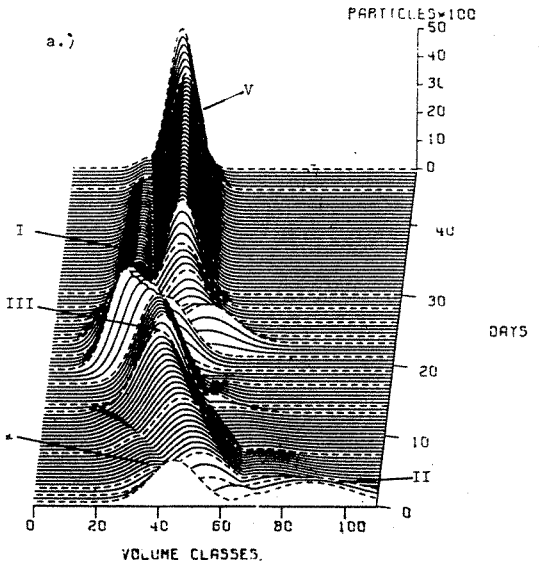


Fig. 1a.

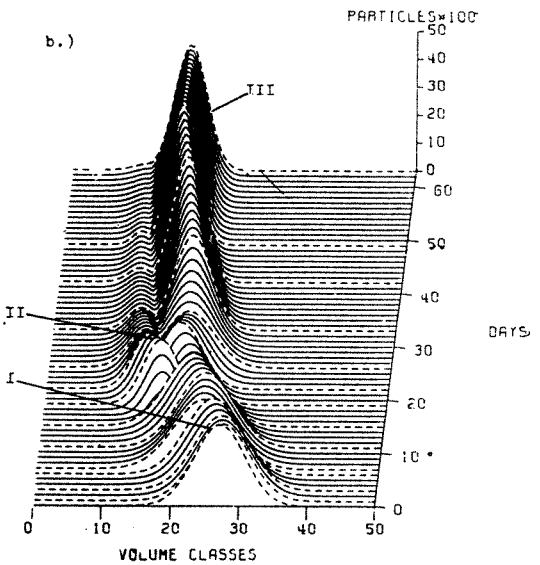


Fig. 1b.

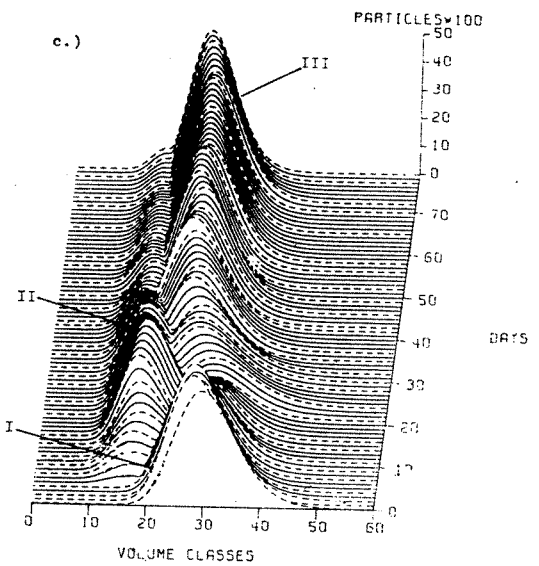


Fig. 1c.

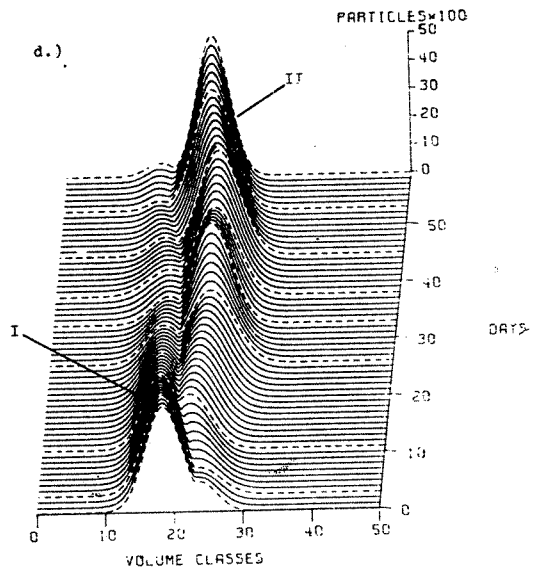


Fig. 1d.

Fig. 1. Development of the erythrocyte volume populations in the peripheral blood of the rat (a), rabbit (b), sheep (c) and guinea pig (d). The dashed line curves show the measured curves and the solid line curves were calculated by linear interpolation in order to increase the three dimensional impression of the plot. The erythrocyte populations appear and disappear in all animals in a continuous development until finaly one erythrocyte population remains in the adult animal.

tions (Fig. 2) is a further proof of non-transformation of the peripheral erythrocytes. It thus seems unlikely that peripheral transformation is the general mechanism for the formation of the different ertythrocyte populations.

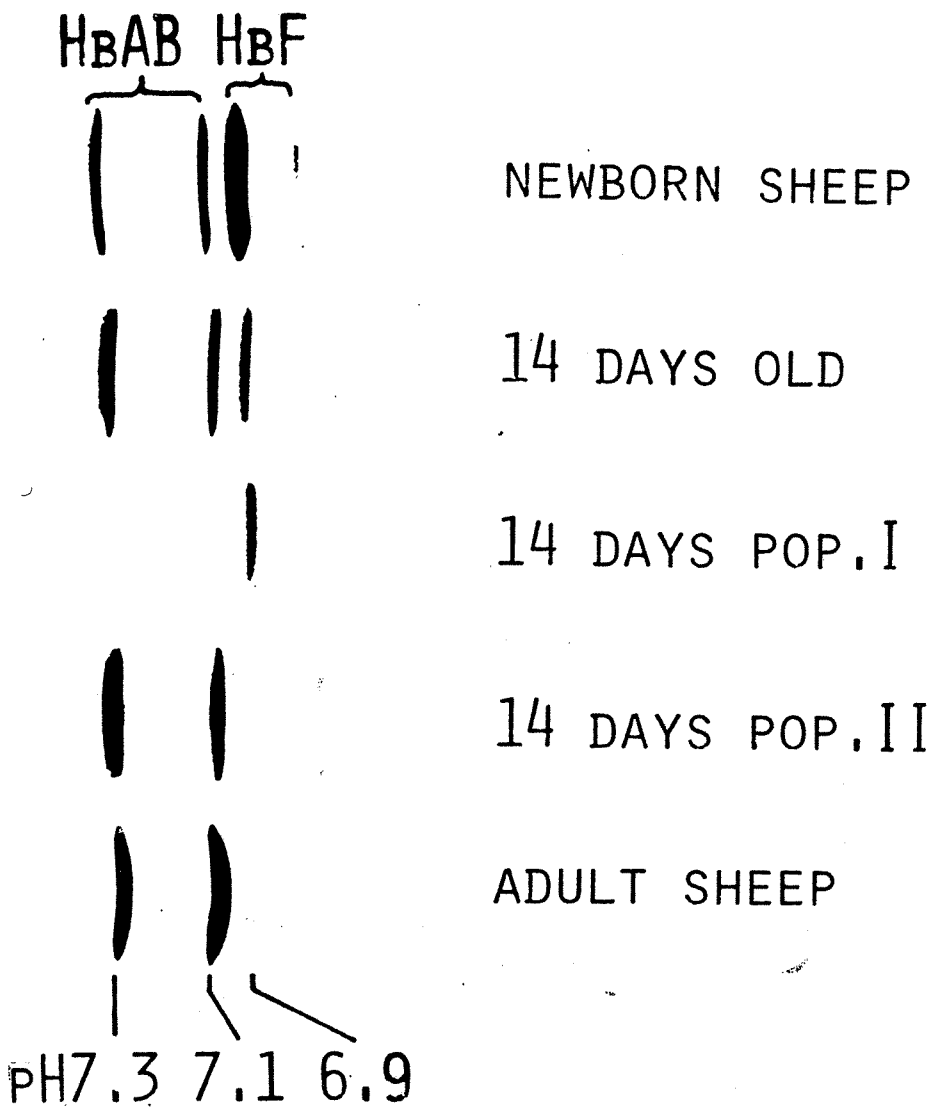


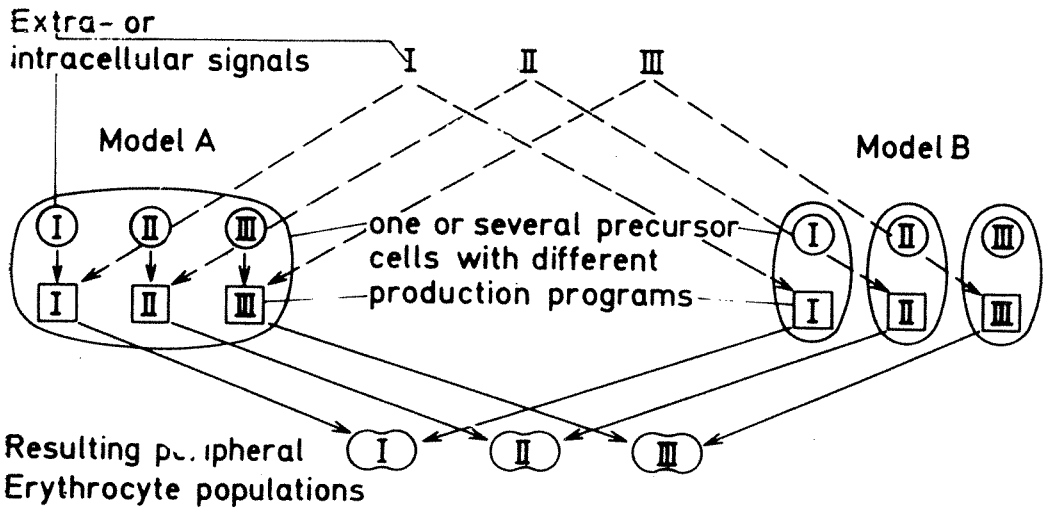
Fig. 2. Isoelectric focusing of fresh sheep erythrocyte lysates from a newborn, a 14 day old and an adult sheep. Haemoglobin F(HbF) is located in the population I erythrocytes (Fig. 1c) and haemoglobin AB (HbAB) in the population II erythrocytes. Population I and II were separated from each other by centrifugal elutriation (elutriator centrifuge, Beckman Co, Fullerton, California) of blood from the 14 day old sheep (11).

It is also of interest to consider the relationship between the different sites of erythrocyte production and the different populations. We believe in the rat that the first two populations are produced by the liver. The populations after birth are simultaneously produced by both the spleen and the bone marrow. This is shown by the fact that surgical removement of the spleen immediately after birth does not change the total number of populations although the kinetics are altered (6). According to preliminary experiments only population IV and V (Fig. 1a) seem to be erythropoietin sensitive (11).

The leukopoietic system is also affected by the changes in the erythropoietic system. The peripheral leukocyte counts in the rat are significantly decreased from day 1-27 after birth during the time of the sequential appearence and disappearence of the erythrocyte populations. The differential counts of the blood leukocytes, the mean volume and the volume distribution curves of the bone marrow and spleen cells and of the peripheral leukocytes also change synchronously with the appearance of the new erythrocyte populations (11). All observations indicate that during the maturation of the haematopoietic system sudden changes occur in the haematopoietic differentiation. These changes concern, at least in the erythropoietic system, several basic cell parameters which are mostly independent from each other. Since both the erythropoietic and the leukopoietic system are affected we believe that the changes of differentiation are initiated at the stem cell level.

Two possible ways for the realization of different gene activation patterns are indicated in Fig. 3. In model A *one* stem cell is capable of realizing different production programs, each with a different set of parameters for the final cells. In model B *several* stem cells are drawn, each of the realising a separate production program. The change from one program to another would be effected in both cases by extra-and/or intracellular signals. Both models could explain the production of different erythrocyte populations with their characteristic parameter patterns as shown, for example, in the sheep (Fig. 3).

We see a particular interest in a more detailed exploration of the above phenomena, because sudden changes of the haematopoietic differentiation in later life may induce severe disease. The causal therapy of such diseases is difficult since the knowledge of the mechanisms which change the haematopoietic differentiation is still



Volume Population	I	II	III
Electrophoretic Mobility	low	intermediate	high
L,M antigen expression	low	low	high
$[K^+]c$	high	intermediate	low
Hemoglobin	HbF	HbC, HbA	HbA,HbC

Fig. 3. Hypothetical concept of the formation of the different erythrocyte populations in the young sheep. Extra-and/or intracellular signals act on *one* (model A) or *several* (model B) haematopoietic stem cells. According to the signal, a different erythrocyte population is produced. Each erythrocyte population is characterised by a particular set of cellular parameters.

incomplete. One of the reasons for this is the lack of convenient model systems in which changes of differentiation can be studied. Physiological changes of differentiation occur normally in the embryonic organism. These mechanisms can, however, only be studied with great difficulty due to the inaccessability of embryos and to the continuous influence of the maternal organism. Since the changes of differentiation described here are postnatal, the are easier to study. We hope that the different cell parameters investigated so far will facilitate the future study of the regulatory circuits which induce changes of differentiation. It could be that in disease such regulatory circuits are interrupted or deviated at specific locations.

Another interesting aspect of the sudden changes of differentiation is that they are present in all mammalian species studied so far. The changes of the differentiation are probably necessary for

the normal development of the organism although the exact reason for the formation of different erythrocyte populations is not evident at present. It seems likely that they are the expression of a normal developmental stage in all mammalian species. The F-cell population in humans (2) may also be a developmental erythrocyte population similar to those described for animals.

This stepwise development of the haematopoietic system may not be confined to mammals. For example discrete erythrocyte populations with different membrane antigens have recently been described during postnatal development of the chicken (1) and population with different haemoglobins have also been described in the tadpole (3). The developmental kinetics of the erythrocyte population EA of the chicken is very similar to that of population II of the sheep, goat and rabbit. Further research is neoded in this area especially to understand the signals which cause the physiological changes of differentiation in postnatal development.

SUMMARY

Several erythrocyte populations of different mean volume are observed in the blood of the rat, mouse, guinea pig, sheep, rabbit and goat during the first three months of life. The populations appear and disappear gradually until finally one erythrocyte volume population remains in the adult animal. As well as having different mean volumes the erythrocyte populations may also vary in their electrophoretic mobility, their antigenic properties, their intracellular Na^+- and K^+-concentration, and their haemoglobin type. The populations arise as a result of multiparameter changes in the haematopoietic differentiation. The leukopoietic system is also affected. It is proposed as a hypothesis that extra-and/or intracellular signals act on one or several different haematopoietic stem cells. The signals switch different production programs on or off. As a result new cell populations in the various cell lines are produced. They replace their predecessors gradually and differ from them in several parameters.

REFERENCES

1. Blanchet, J.P.: Developmental changes of chic erythrocyte membrane antigens. Exp. Cell Res. *104*, 1 (1976).
2. Boyer ,S.H., Belding, T.K., Margolet, L., Noyes, A.N.: Restriction of fetal

hemoglobin to a few erythrocytes (T-cells) in normal human adults. Science *188*, 361 (1975).

3. Broyles, R.H., Deutsch, M.J.: Differentiation of red blood cells in vitro. Science *190*, 471 (1975).
4. Blunt, M.H., Huisman, T.H.J.: The haemoglobins of the sheep. In: The blood of the sheep. Editor M.H. Blunt, Springer Verlag, Berlin 1975, p. 155-183.
5. Mitteilungen der IV. Converence on automated cytology. Asilomar 1975. J. Histochem. Cytochem. *24*, 211 (1976).
6. Hanser, G., Valet, G., Boss, N., Ruhenstroth-Bauer, G.: Origin and regulation of the different erythrocytes volume populations in the newborn rat, XV. Congress Int. Soc. Hematology, Jerusalem 1974, p. 318 abstr.
7. Kachel, V.: Basic principles of electrical sizing of cells and particles and their realization in the new instrument «Metricell». J. Histochem. Cytochem. *24*, 211 (1976).
8. Lindsay, D.B., Leat, W.M.F.: Carbohydrate and lipid metabolism, in: The blood of the sheep. Editor M.H. Blunt, Springer Verlag, Berlin 1975, p. 45-62.
9. Park, B.H., Good, R.A.: I. Principles of basic immunology, the influence of nutritional deficiency and ageing. In: Principles of modern immunebiology. Lea and Febiger, Philadelphia 1974, p. 189-199.
10. Tucker, E.M.: Genetic markers in the plasma and red blood cells. In: The blood of the sheep. Editor M.H. Blunt, Springer Verlag, Berlin 1975, p. 123-153.
11. Valet, G. et al. in preparation.
12. Valet, G., Franz, G., Lauf, P.K.: Different red cell populations in newborn, genetically low potassium sheep: relation to haematological, immunological and physiological parameters and to erythropoietic differentiation (manuscript submitted).
13. Valet, G., Hofmann, H., Ruhenstroth-Bauer, G.: The computer analysis of volume distribution curves, demonstration of two erythrocyte populations of different size in the young guinea pig and analysis of the mechanism of immune lysis of cells by antibody and complement. J. Histochem. Cytochem. *24*, 231 (1976).
14. Valet, G., Schindler, R., Hanser, G., Ruhenstroth-Bauer, G.: Several erythrocyte populations of different mean volume in the young sheep and rat with different electrophoretic mobilities. Third meeting European and African Division Int. Soc. Haematology, London 1975, abstr. 18 : 02.
15. Valet, G., Hanser, G., Metzger, H., Ruhenstroth-Bauer, G.: Several erythrocyte volume populations in the blood of the newborn rat, mouse, guinea pig and in the human fetus. XV. Congress Int. Soc. Haematology, Jerusalem 1974, p. 317 abstr.
16. Valet, G., Metzger, H., Kachel, V., Ruhenstroth-Bauer, G.: Die Volumenverteilungskurven von Rattenerythrozyten nach Röntgenganzkörperbestrahlung. Blut *24*, 274 (1972).
17. Valet, G., Metzger, H., Kachel, V., Ruhenstroth-Bauer, G.: Der Nachweis verschiedener Erythrozytenpopulationen bei der Ratte. Blut *24*, 42 (1972).