Four Types of Presumptive Hemopoietic Stem Cells in the Human Fetal Liver

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Summary. Presumptive hemopoietic stem cells in the human liver obtained from 109 embryos 28 to 49 days after ovulation and 76 fetuses between 8 and 22 weeks of ovulation were investigated by light and electron microscopy.

Presumptive hemopoietic stem cells in the human embryonic liver are concluded to be a series of cells that show a variegated ultrastructure. They are classified into four subtypes (type I, II, III and IV). Presumptive hemopoietic stem cells of type I are thought to differentiate from the undifferentiated mesenchymal cells that are derived from the septum transversum. Presumptive stem cells of type I, II and III transitorily appear in the liver during the early stage of hepatic hemopoiesis, and cannot be detected in late stages. With the development of the fetus, presumptive stem cells of the type IV, however, gradually increase in number. The cells of megakaryocytic, granulocytic and erythrocytic lineages originate from the presumptive stem cells of type II in the early stage of hepatic hemopoiesis, whereas the cells of the three lineages originate from the presumptive stem cells of type IV in the late stage. The presumptive hemopoietic stem cells of type IV are surmised as corresponding to the pluripotent hemopoietic stem cells (CFU-S) in laboratory animals or pluripotent hemopoietic progenitors in human bone marrow (CFU-mix).

Experimental investigations of the colony forming units in hemopoiesis have verified the existence of pluripotential hemopoietic stem cells (CFU-S) in laboratory animals (Till and McCulloch, 1961; Becker, McCulloch and Till, 1963). Recent studies of mixed colony formation have revealed the presence of human pluripotent hemopoietic progenitors in the human bone marrow (CFU-mix) (Fauser and Messner, 1978, 1979). The ultrastructure of CFU-S is considered to resemble that of small lymphocytes (Bekkum et al., 1971; Dicke, Noord and Bekkum, 1973; Visser et al., 1977; Goldschneider et al., 1980).

In our previous papers, it was reported that the pluripotent hemopoietic stem cells in the liver in the early stage of hepatic hemopoiesis differed in ultrastructure from those in late stage of hepatic hemopoiesis (Emura, Sekiya and Ohnishi, 1983a, b, c).

In this context, the present investigation was conducted to elucidate the origin and the ultrastructure of the presumptive hemopoietic stem cells in human embryonic and fetal liver.
MATERIALS AND METHODS

The hepatic tissues examined were from 109 human embryos 28 to 49 days after ovulation and 76 human fetuses at 8 to 22 weeks of ovulation, all being obtained by legal abortion from healthy women.

Light microscopy: Eighteen embryos between 4 to 24 mm crown rump (C. R.) length (estimated age: 30 to 49 days after ovulation) and hepatic tissue of 34 fetuses (50 to 154 days of ovulation) were fixed in 10% neutral formalin and then embedded in paraffin. Every fifth serial section was stained with hematoxylin-eosin, and the rest were subjected to a silver impregnation, periodic acid-Schiff reaction and naphthol AS-D chloroacetate esterase.

Transmission electron microscopy: Ninety-one embryos, including a 21 somite embryo of 3 mm C. R. in length (estimated age, 27±1 days), and hepatic tissues of 76 fetuses were fixed as soon as possible in fixative, after legal abortion, which consisted of 2% glutaraldehyde in 0.1 M phosphate buffer solution, pH 7.5, at 4°C for 2 hrs. In the fixative, the embryos and livers were divided into small pieces using a dissection microscope. The tissue pieces were then rinsed in 0.1 M phosphate buffer solution, pH 7.5, and postfixed in 1% osmium tetroxide at 4°C for 2 hrs. All the specimens were dehydrated and embedded in Epon. Ten sets of 30–40 serial ultrathin sections were routinely prepared at an interval of 15–20 μm from a block of each case. Ultrathin sections were counterstained with uranyl acetate and lead citrate. The sections were examined with Hitachi HS-9 electron microscopy.

RESULTS

I. Hemopoietic organs and hemopoiesis

A. Yolk sac, bone marrow, spleen, lymphnodes and thymus

The yolk sac was a sole hemopoietic organ during the earliest stage of fetal development. Most hemopoietic cells in the yolk sac were erythroid.

Within 7 weeks of ovulation, no hemopoiesis was observed in the cartilaginous rudiment of bones. Bone marrow hemopoiesis first appears in the claviculas of a fetus of 10 weeks of ovulation and then in femurs, humeruses of a fetus of 11 weeks of ovulation (SEKIYA, 1982).

The spleen appeared in embryos of about 10 mm C. R. in length as a localized condensation of mesodermal cells in the dorsal mesogastrium. Hemopoietic cells, however, are first observed in the spleen of an embryo after 15 weeks of ovulation (HAMILTON and MOSSMAN, 1976; FUKUDA, 1982).

Until 7 weeks of ovulation, no small lymphocytes were identified in a densely packed epithelial primordia of the thymus. Small lymphocytes (thymocytes) appear among more loosely arranged epithelial cells of the thymus of an embryo of 35 mm C. R. length (estimated ovulation age: 9th week) (HAMILTON and MOSSMAN, 1976).

No lymph node was noticed in an embryo of 13 mm C. R. in length (estimated ovulation age: 40 days). Lymph nodes are developed by the aggregation of lymphocytes in the mesenchyme. The aggregations are first found in 30 mm embryos (55 days) (HAMILTON and MOSSMAN, 1976).
B. Hepatic parenchyma

The hepatic parenchyma has been described as developing from the cephalic end of the hepatic diverticulum (DuBois, 1963). Within the first month, the hepatic parenchymal cords were irregular and hepatocytes were intermingled with the undifferentiated mesenchymal cells derived from the septum transversum. Numerous mitotic figures were found among hepatocytes and mesenchymal cells, and primitive erythroblasts of the yolk sac origin were found in the sinusoids, but no hemopoietic cells were found in the extravascular spaces.

A small number of types I and II presumptive hemopoietic stem cells were first identified in the hepatic parenchyma of an embryo of 6.2 mm C. R. length (estimated ovulation age: 33 days) (Fig. 1). With the embryo's development, erythroid cells quite rapidly increased in number, the number of megakaryocytic and granulocytic cells were far fewer than that of erythrocytic cells. Presumptive hemopoietic stem cells type I gradually reduced in number and could not be detected in any later stage.

A very small number of presumptive hemopoietic stem cells type III and IV were first found in the hepatic parenchyma of an embryo of a 13 mm C. R. length (estimated ovulation age: 40 days) (Fig. 2). Presumptive hemopoietic stem cells type III increased in number transitorily, but these cells gradually decreased in number and could not be detected in the later prosperous stage of hepatic hemopoiesis. Presumptive hemopoietic stem cells type IV, however, increased in number in the hepatic parenchyma. Forty days after ovulation, hemopoiesis in the hepatic parenchyma seemed established.

![Fig. 1. Hepatic parenchyma of an embryo, about 34 days after ovulation. Presumptive hemopoietic stem cells type I or II are found in the extravascular space (large arrow). Undifferentiated mesenchymal cells are scattered among hepatocytes (small arrows). ×1,200](image-url)
C. Ductus venosus

During the early stage of hepatic development, many undifferentiated mesenchymal cells were found between the endothelium of the ductus venosus and hepatic parenchyma, and hepatocyte showed direct and intimate contact with the adjacent mesenchymal cells. Within 40 days of ovulation, mitotic figures were frequently found among the mesenchymal cells around the ductus venosus. No hemopoietic cells, however, were found there.

A small number of presumptive hemopoietic cells type I and II were first discovered around the ductus venosus of an embryo 14 mm C. R. in length (estimated ovulation age, about 40 days). Unlike the changes in hepatic parenchyma, numerous presumptive hemopoietic stem cells type III and the cells of granulocytic lineage rapidly increased simultaneously in number (Fig. 3). Presumptive hemopoietic stem cells type IV were identified around the ductus venosus of an embryo of 15.5 mm C. R. length (estimated ovulation age, about 43 days). With the development of embryos, presumptive hemopoietic stem cells type IV increased in number, however presumptive hemopoietic stem cells type I, II and III gradually reduced in number and could not be detected in later stages. Seven weeks after ovulation, granulopoiesis around the ductus venosus seemed established. Neither erythrocytic nor megakaryocytic hemopoiesis developed there.
D. Characteristics of presumptive hemopoietic stem cells

Presumptive hemopoietic stem cells type I, II and III were observed exclusively in the extravascular spaces. Presumptive hemopoietic stem cells type IV were also found solely in the extravascular spaces in early stage of hepatic hemopoiesis, but in later stages these cells were found in the extravascular spaces and in sinusoidal lumina as well.

Mitotic figures were frequently found among presumptive hemopoietic stem cells type I and II, however, mitosis of both type III and IV was rarely detected.

We could not notice any morphological differences between the presumptive hemopoietic stem cells in the hepatic parenchyma and those around the ductus venosus.

E. Undifferentiated mesenchymal cells (Fig. 4)

These cells were provided with many dendritic cytoplasmic processes, and desmosome-like attachments were detected between these cells or these cells and hepatocytes. The nucleus occupied the greater part of a cell and contained one or more prominent nucleoli. The chromatin was finely dispersed in the nucleus. Nuclear pores were moderate in number. The perinuclear cisterna was slightly dilated. Moderately dilated and branched cisternae of rough endoplasmic reticulum were filled with an amorphous material. Glycogen particles appeared in the cytoplasm. Polyribosomes, single ribosomes and mitochondria were small in number. The Golgi apparatus was composed of lamellar cisternae and small vesicles. Lysosomal granules or phagolysosomes were rarely observed. A pair of centrioles were usually found. Cilium was

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Fig. 3. Presumptive hemopoietic stem cells type III and the cells of granulocytic series are observed among the mesenchymal cells around the ductus venosus of an embryo, about 45 days after ovulation. ×2,400
occasionally noticed. No basement membrane was detected along the cell membrane.

Some cells as shown in Figure 5 were often found among hepatocytes together with presumptive stem cell type I, II and undifferentiated mesenchymal cells. These cells had irregular cytoplasmic projections. The large nucleus contained a prominent nucleoli. The chromatin was finely dispersed in the nucleus and nuclear pores were numerous. The polyribosomes and mitochondria increased in number in comparison with the undifferentiated mesenchymal cells. On the contrary, moderately dilated cisternae of rough endoplasmic reticulum and glycogen particles were rarely found. The Golgi apparatus was composed of short lamellar cisternae and small vesicles. Lysosome-like granules were occasionally found and a pair of centrioles were located near the Golgi apparatus.

II. Presumptive hemopoietic stem cells

In this paper, the presumptive hemopoietic stem cells are classified into four subtypes. The cells that were described as undifferentiated mononuclear cells in our previous papers (EMURA, 1978; EMURA, SEKIYA and OHNISHI, 1980) correspond to presumptive stem cells type I in this paper, and in the same way, polyribosome-rich undifferentiated mononuclear cells to type II, large lymphoid mononuclear cells to type III and small lymphoid mononuclear cells to type IV.

A. Presumptive hemopoietic stem cells type I (Fig. 6)

The cells of this group were uniform in ultrastructure. These cells were round or oval.
in shape, ranging from 9 to 11 μm in the largest diameter. The slightly indented nucleus occupied the greater part of the cell. Small but prominent nucleoli were present in the nucleus and the chromatin was finely dispersed. Nuclear pores were numerous. The narrow cytoplasm contained a small number of polyribosomes and mitochondria. Short cisternae of rough endoplasmic reticulum were occasionally observed. Lysosome-like granules were rarely detected but glycogen particles were hardly found. The Golgi apparatus was composed of a few cisternae and small vesicles. A pair of centrioles were found between the nucleus and the Golgi apparatus. Polyribosomes of these cells were more abundant than those of undifferentiated mesenchymal cells and the cytoplasm was darker than that of undifferentiated mesenchymal cells.

B. Presumptive hemopoietic stem cells type II (Fig. 7-9)
The cells of this group showed a variegated ultrastructure. These cells ranged in size from 9 to 15 μm. They showed a high nucleocytoplasmic ratio. One or two prominent nucleoli were present in the slightly indented nucleus. The chromatin of the cells shown in Figures 7 and 8 was finely dispersed in the nucleus and the nuclear pores were numerous, whereas the chromatin of the cells shown in Figure 9 showed a very slight condensation and the nuclear pores were reduced in number. Polyribosomes and mitochondria increased in number. Polyribosomes of the cell shown in Figure 8 were more abundant than those of the cell shown in Figure 7. A few of lysosome-like granules were rarely observed. Cisternae of rough endoplasmic reticulum were rarely found. The Golgi apparatus was composed of lamellar cisternae and small vesicles.
A pair of centrioles were usually situated between the nucleus and the Golgi apparatus. A desmosome-like attachment found between hepatocytes and the immature cells of erythrocytic lineage was not detected between the cells of this group and hepatocytes or mesenchymal cells. No specific structures characteristic of the cells of granulocytic, erythrocytic, monocytic or megakaryocytic lineages were detected in the cytoplasm of these cells.

C. Presumptive hemopoietic stem cells type III (Fig. 10–12)

The cells of this group were not uniform in ultrastructure. The size of these cells ranged from 13 to 7 μm. The nucleus was irregular in shape and nuclear pores were small in number. Significant nuclear indentations were frequently found. The nucleolus was less prominent. The chromatin showed reticular clumping to a variegated degree. The chromatin of smaller cells was condensed more coarsely than that of larger ones. The cytoplasm of the larger cells of this group had a moderate number of polyribosomes and mitochondria. But with reduction of cell size, the cytoplasmic rim became narrow and all organelles became gradually reduced in number and size.

Fig. 6. A presumptive hemopoietic stem cell type I among the hepatocytes of an embryo, about 34 days after ovulation. The nucleus occupies the greater part of the cell. The chromatin is finely dispersed in the nucleus. ×11,000

Fig. 7. A presumptive hemopoietic stem cell type II in the intercellular spaces of an embryo, about 38 days after ovulation. The mitochondria and polyribosomes are increased in number. ×11,000

Fig. 8. A presumptive hemopoietic stem cell type II in the extravascular space in the hepatic parenchyma of an embryo, about 35 days after ovulation. Polyribosomes are markedly increased in number. ×10,000
Fig. 7 and 8. Legends on the opposite page.
The small Golgi apparatus was composed of a few cisternae and small vesicles. A pair of centrioles were situated between the Golgi apparatus and the nucleus. Cisternae of rough endoplasmic reticulum were small in number.

D. Presumptive hemopoietic stem cells type IV (Fig. 13)

The cells of this group were homogeneous in ultrastructure. These cells were round in shape and ranged from 6 to 8 μm in diameter. Usually a small nucleolus was found in the slightly irregular nucleus. The chromatin showed coarse accumulations. A few nuclear pores were found. A small number of single ribosomes were observed in the narrow cytoplasm. Mitochondria had a frequency of 1 to 3 per section. Cisternae of rough endoplasmic reticulum were rarely found. The small Golgi apparatus was formed by a few cisternae. A pair of centrioles were observed near the Golgi apparatus. A few small cytoplasmic projections were provided on the cell surface.

DISCUSSION

Multipotential hemopoietic stem cells have been studied morphologically by many investigators. Recently, using an experimental approach of colony forming units, much knowledge has been accumulated about hemopoietic stem cells. However, the ultrastructures of hemopoietic stem cells in human beings have not been fully understood and there are gaps between the results obtained by morphological investigations and
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The present investigation revealed the precise ultrastructure of presumptive hemopoietic stem cells in human embryonic and fetal liver. The presumptive hemopoietic stem cells type I described here are surmised to coincide with the undifferentiated mononuclear cells detected by FUKUDA (1973) in human embryonic liver. These cells can be readily distinguished from the other immature hemopoietic cells in the fetal liver because of far less abundant cytoplasmic organelles and the more finely dispersed nuclear chromatin of these cells.

Presumptive hemopoietic stem cells type II are surmised to correspond to the transitional cells (FUKUDA, 1974) or the stem cells (ZAMBONIE, 1965) both found in human embryonic liver.

Presumptive stem cells type III should be distinguished from the late hepatic erythroid progenitor cells, the late hepatic myeloid progenitor cells and the late hepatic megakaryoblasts in human fetal liver (EMURA, SEKIYA and OHNISHI, 1983a, b, c). However, the former cells are found in the liver before the presumptive hemopoietic stem cells type IV increase in the liver, and these former cells can be distinguished from the latter cells because the cytoplasm of the latter cells contain far abundant organelles, such as mitochondria and free ribosomes than that of the former cells.

Presumptive hemopoietic stem cells type IV can be easily distinguished from the other immature hemopoietic cells in human embryonic liver.

The above mentioned four types of presumptive stem cells are not able to be clas-

Fig. 10. A presumptive hemopoietic stem cell type III in the intercellular space of the mesenchymal cells around the ductus venosus of an embryo, about 45 days after ovulation. The chromatin shows slight reticular condensation. The polyribosomes and mitochondria are reduced in number. ×11,500
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sified as the immature cells of one of various hemopoietic series because we cannot
detect any characteristic ultrastructures of erythrocytic, megakaryocytic, monocytic
and granulocytic lineages in the cytoplasm of these cells.

STITES, CARR and FUDENBERG (1974) reported that hemopoietic cells in the liver of
a fetus, about 60 days after ovulation (C. R. length 34 mm) had the ability to respond to
allogeneic lymphocytes in the mixed lymphocyte reaction, so lymphocytes may origi-
nate from presumptive stem cells type IV. The cells of the presumptive stem cells
shown in this paper, however, are not considered to be the cells of lymphocytic series,
because neither bone marrow nor lymph nodes are found and no lymphocytes are de-
tected in the primordia of the thymus and the spleen of an embryo of 40 days after
ovulation, in which all four types of presumptive stem cells are found.

From our ultrastructural investigation of megakaryocytic, granulocytic and ery-
throcytic series in human embryonic liver (EMURA, SEKIYA and OHNISHI, 1983a, b, c), it
was concluded that the multipotential stem cells in the liver at the early stage of hepatic
hemopoiesis were about 14 pm in average diameter. The nucleus had large nucleoli
and the chromatin was finely dispersed in the nucleus. The nuclear pores were numer-
ous. Organelles, especially polyribosomes and mitochondria were abundant in the
cytoplasm. The presumptive stem cells type II shown in Figure 8 are well coincident
with the above mentioned montage in ultrastructure. In the same way, it was believed
that the hemopoietic stem cells in late stage of hepatic hemopoiesis were about 8 pm in
average diameter. The chromatin showed coarse condensation and the nucleus was

Fig. 11. A presumptive hemopoietic stem cells type III among the mesenchymal cells around the
ductus venosus of an embryo, about 45 days after ovulation. The cell decreases in size and
the chromatin shows reticular accumulation. The organelles are markedly reduced in num-
ber and size. ×12,000
provided with a small nucleolus and a small number of nuclear pores. Organelles were scarce in the narrow cytoplasm. The presumptive stem cells type IV were identical in ultrastructure to the picture.

Since the four types of presumptive stem cells appear in regular sequence in the liver, and since it is possible to recognize a continuity of ultrastructure from the presumptive hemopoietic stem cells type I to IV, it seems logical to draw the following three conclusions: 1) the multipotential hemopoietic stem cells in human embryonic and fetal livers are not be a group of cells that show uniform ultrastructure but to be a series of cells that show variegated ultrastructure. 2) Presumptive hemopoietic stem cells type I, II and III occur transitorily in the liver during the early stage of hepatic hemopoiesis, and cannot be detected in late prosperous stages of hepatic hemopoiesis. However, presumptive hemopoietic stem cells type IV originate from the presumptive stem cells type I, II and III. The presumptive stem cells type IV gradually increase in number with the development of the fetus, last in fetal liver. 3) the cells of granulocytic, megakaryocytic and erythrocytic lineages originate from the presumptive stem cells type II in early stage of hepatic hemopoiesis and those of the three lineages from the presumptive stem cells type IV in the late stage of hepatic hemopoiesis. Our conclusion is summarized schematically in Figure 14.

Proportions of the four types of presumptive hemopoietic stem cells both in hepatic parenchyma and around the ductus venosus are diagrammatically shown in Figure 15. In comparison with the changes in hepatic parenchyma, all four types of presumptive
stem cells increased simultaneously around the ductus venosus. Since the embryo is free from bacterial infection during the intrauterine period, most presumptive stem cells, type I and II, around ductus venosus are considered to differentiate to presumptive stem cells type III and IV. On the other hand, the rapidly developing embryo usually requires an increasing number of erythrocytes, so most presumptive stem cells, type I and II, that occurred in hepatic parenchyma are thought to differentiate into the cells of erythrocytic or megakaryocytic lineages.

There has been a long discussion concerning the possibility of hepatic hemopoietic stem cells originating in the liver, from endodermal cells (Thomas and Yoffey, 1961, 1964), reticuloendothelial cells (Karrer and Cox, 1961; Boyd, 1970) or from other mesenchymal cells (Zamboni, 1965; Rifkind, Chu and Epler, 1969; Fukuda, 1974) or come from the yolk sac via the blood stream (Barnes and Loutit, 1967; Loutit, 1968; Metcalf and Moore, 1971). Recent experimental investigation has skillfully demonstrated that the stem cells of avian arose within the embryo and did not originate from the yolk sac (Dieterlen-Lievre, Beaupain and Martin, 1979).

The presumptive stem cell type I, II and III were found exclusively in the extravascular spaces, and no cell was found passing through the sinusoids to the Disse’s spaces or in the sinusoidal lumina. The presumptive stem cells type IV were frequently detected in sinusoidal lumina in the late stage of hepatic hemopoiesis, but during the early stage, these cells were also observed solely in the extravascular spaces.

In addition, we could often find odd cells, as shown in Figures 5, in the intercellular

Fig. 13. A presumptive hemopoietic stem cell type IV in the intercellular space of the mesenchymal cells around the ductus venosus of an embryo, about 47 days after ovulation. The chromatin shows a coarse condensation. The cytoplasm contains a small Golgi apparatus, a centriole and a small amount of single and polyribosomes. × 12,000
Four Types of Human Presumptive Stem Cells

Fig. 14. Differentiation of blood cells. Model of hematopoiesis in human embryonic and fetal liver. UMeC: undifferentiated mesenchymal cell, I: presumptive hemopoietic stem cell type I, II: presumptive hemopoietic stem cell type II, III: presumptive hemopoietic stem cell type III, IV: presumptive hemopoietic stem cell type IV, Myel. Pro. C.: myeloid progenitor cell, My. Bl.: myeloblast, Neut: neutrophile, Mg. Pro. C.: megakaryocytic progenitor cell, Mg. Bl.: megakaryoblast, Plat.: platelet, Ery. Pro. C.: erythrocytic progenitor cell, Pr. Ery. Bl.: proerythroblast, Ery.: erythrocyte.

Fig. 15. Proportions of the four types of presumptive hemopoietic stem cells. I: presumptive hemopoietic stem cell type I, II: presumptive hemopoietic stem cell type II, III: presumptive hemopoietic stem cell type III, IV: presumptive hemopoietic stem cell type IV. Presumptive stem cells type II in the hepatic parenchyma closely resemble immature cells of erythrocytic or megakaryocytic lineages, so the presumptive stem cells type II may linger in the fetal liver and differentiate into erythrocytic or megakaryocytic series for a while.
spaces of the hepatocytes and among the undifferentiated mesenchymal cells around the ductus venosus, at the initial stage of hepatic hemopoiesis. These cells were provided with irregular extensions of cytoplasm and were similar in contour to undifferentiated mesenchymal cells. Organelles, especially polyribosomes of these cells were more abundant than those of the undifferentiated mesenchymal cells. Dilated and branched cisternae of rough endoplasmic reticulum and glycogen particles that were characteristic of undifferentiated mesenchymal cells were far fewer in these cells. From these findings, we speculate that these cells are in stages of differentiation between undifferentiated mesenchymal cells and the presumptive hemopoietic stem cells type I.

During the process of the development of the primitive liver, many mesodermal mesenchymal cells of the septum transversum were caught between the hepatocytes or in the subendothelial spaces. Therefore, the presumptive hemopoietic stem cells type I described here are thought to originate from undifferentiated mesenchymal cells derived from the septum transversum.

Experimental investigations of colony-forming units verified the existence of pluripotential hemopoietic stem cells (CFU-S) in experimental animals (Till and McCulloch, 1961; Becker, McCulloch and Till, 1963) and in human (CFU-mix) (Fauser and Messner, 1978, 1979). It is reported that the candidate stem cells (CFU-S) are 7–10 μm in diameter (Bekkum et al., 1971). Using the velocity sedimentation method, CFU-S were classified into two subtypes (Worton et al., 1969a) and the presumptive ultrastructures of the two subsets of CFU-S were reported to show lymphoid ultrastructure (Visser et al., 1977; Goldschneider et al., 1980). The greater part of CFU-S is in a resting stage (Go) of mitosis (Becker et al., 1965). The size of CFU-mix is thought to be equal to that of CFU-S (Worton et al., 1969b; Hara and Ogawa, 1977, 1978). Presumptive hemopoietic stem cells type IV resemble candidate pluripotent hemopoietic stem cells (CFU-S) in the ultrastructure. Furthermore, the greater part of the cells of type IV is surmised to be in the Go stage of mitosis because the mitoses of these cells were hardly found. From these findings we surmised that presumptive hemopoietic stem cells type IV correspond to a CFU-S or CFU-mix.

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