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Haemopoiesis in the human yolk sac

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INTRODUCTION

Since Wolff (1896) reported the presence of blood islands in chicken yolk sacs, ontogenic development of haemopoietic tissues has been studied in various mammalian species, for example, the bat (Van der Stricht, 1899), the cat, dog, guinea-pig and mouse (Maximow, 1909), the pig (Jordan, 1916), the rabbit (Maximow, 1909; Van der Stricht, 1899), the rat (Maximow, 1909; Block, 1946) and the shrew (Hubrecht, 1894). These studies revealed that blood cell formation occurs first in the mesoderm.

In the human embryo, haemopoiesis appears first in the yolk sac. Jordan (1907) suggested that the sole function of the human yolk sac was differentiation of mesoderm into blood islands leading to the formation of embryonic blood cell progenitors. Bloom (1940) reported that haemopoiesis in the human yolk sac appeared first in the mesoderm. In contrast, electron microscopic studies reported by Fukuda (1973, 1978) showed the presence of erythroblastic islands in the endodermal layer of human yolk sacs.

In the present study, light and electron microscopic examinations were made of human yolk sacs in various stages of development in order to elucidate the site of haemopoiesis in them.

MATERIALS AND METHODS

Materials were obtained from consenting pregnant patients who had undergone hysterectomy because of uterine myoma or cervical cancer and from those who had undergone legal abortion during the past 8 years. Yolk sacs were obtained from a total of 27 cases, comprising one at the fourth week of pregnancy, 3 at the fifth week, 4 at the sixth week, 9 at the seventh week, 5 at the eighth week, 2 at the ninth week, 2 at the tenth week and 1 at the eleventh week, respectively. The duration of pregnancy was estimated from the menstrual history and the crown–rump length of the embryos.

For light microscopy, the extirpated yolk sac tissues were fixed in 95 % ethanol or 10 % buffered formalin. Paraffin sections were stained with haematoxylin and eosin, Giemsa or the periodic acid–Schiff reaction. For electron microscopy, small pieces of tissue were fixed in 2·5 % glutaraldehyde in 0·1 M cacodylate buffer, pH 7·4, for one hour, and were postfixed in 0·2 % osmium tetroxide in the same buffer for one hour. They were embedded in Epon 812 after dehydration in a graded ethanol series. Ultrathin sections were prepared with the aid of an LKB 2088 Ultratome V and stained with uranyl acetate and lead citrate. Adjacent semithin sections were also prepared from the Epon-embedded material, and these were stained with toluidine blue for light microscopic identification of the cells observed by electron microscopy.

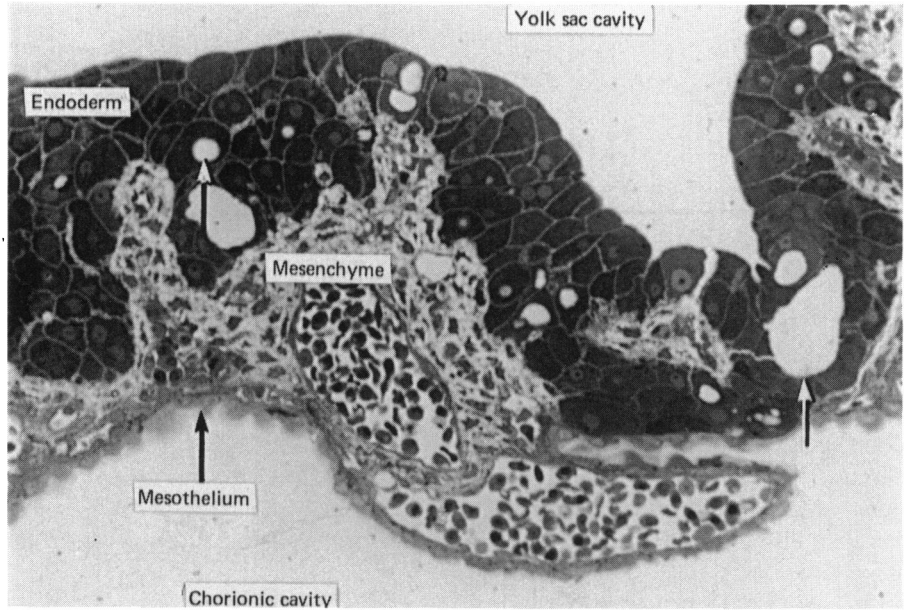

Fig. 1. Endodermal tubules and intracellular tubules are observed in the endodermal layer (arrows). 8th week of pregnancy. Toluidine blue. ×200.

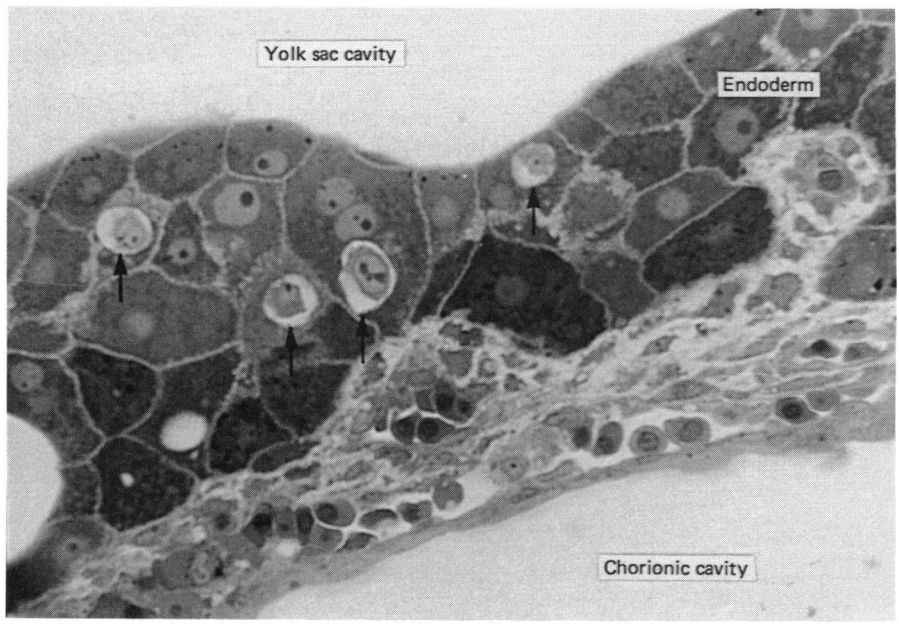

Fig. 2. Blood cells with large nuclei are observed in the endodermal layer and in intracellular tubules (arrows). Nucleated erythrocytes are observed in the mesenchyme and within blood vessels. 6th week of pregnancy. Toluidine blue. ×300.

RESULTS

Light microscopy

The human yolk sac is present in the chorionic cavity until approximately the twelfth week of pregnancy. Light microscopy showed that the yolk sac wall, which was about 200 to 500 μm in thickness, was made up of three layers. These were the endodermal layer, consisting of columnar cells arranged in a flagstone-like arrangement facing the yolk sac cavity; the mesothelial layer, consisting of one to two layers of flat cells facing the chorionic cavity and covering the yolk sac and vitelline duct, and the mesenchymal layer, which consisted of stellate cells and was situated between the endodermal and mesothelial layers. In the endodermal layer, as shown in Figures 1 and 2, several endodermal cells were arranged to form tubular structures (endodermal tubules), and formation of tubular structures was also encountered within the cytoplasm of the endodermal cells (intracellular tubules). In the early stages of pregnancy, the endodermal tissues were found to occupy the major part of the yolk sac walls, but the proportion decreased as pregnancy advanced. On the other hand, the proportion of mesenchymal tissue increased during pregnancy and occupied more than half of the yolk sac wall at the seventh to eighth week. Nucleated blood cells in various stages of differentiation and maturation were observed in the endodermal and mesenchymal layers. Mature nucleated and anucleate red blood cells were found only within blood vessels (Figs. 2, 3). Blood cells exhibiting mitosis were seen scattered in the endodermal layer. In the early stages of pregnancy, blood cells and blood islands were detected in the endodermal layer and also between the endodermal and mesothelial layers (Figs. 4, 5). They later disappeared in the endodermal layer but, conversely, became conspicuous in the mesenchyme as the pregnancy advanced (Fig. 6). In general, they became inconspicuous after the eighth week of pregnancy. In the endodermal tubules and also in the intracellular tubules, blood cells with large nuclei were observed (Fig. 2).

Light microscopic findings in the vitelline duct (yolk duct) walls

The vitelline duct walls contained blood vessels and endodermal tubules, both covered by mesothelial tissues. The mesenchymal tissues observed between the endodermal and mesothelial layers in the yolk sac walls were not observed in the vitelline duct walls at any stage of pregnancy (Fig. 7).

Electron microscopy

Endodermal cells showed numerous slender cytoplasmic protrusions on the cell surface which were connected to each other by desmosomes. The nuclei of the cells were ovoid in shape and had one or two nucleoli. As shown in Figure 8, the cytoplasm was abundant and rich in parallel cisternae of rough endoplasmic reticulum, glycogen granules, coated vesicles and mitochondria. In contrast, the mesenchymal cells appeared to be connected to each other by many irregular cytoplasmic protrusions, and their cytoplasm was rather scanty. However, the cytoplasmic organelles were relatively well developed, particularly in the cells located in the vicinity of the endodermal layers, and they resembled those in the endodermal cells. The nuclei were irregular in shape and showed condensed chromatin along the nuclear membranes (Fig. 9). Haemocytoblasts were seen scattered in the endodermal tubules and also in the endodermal cells. Haemocytoblasts showed large globular nuclei con-

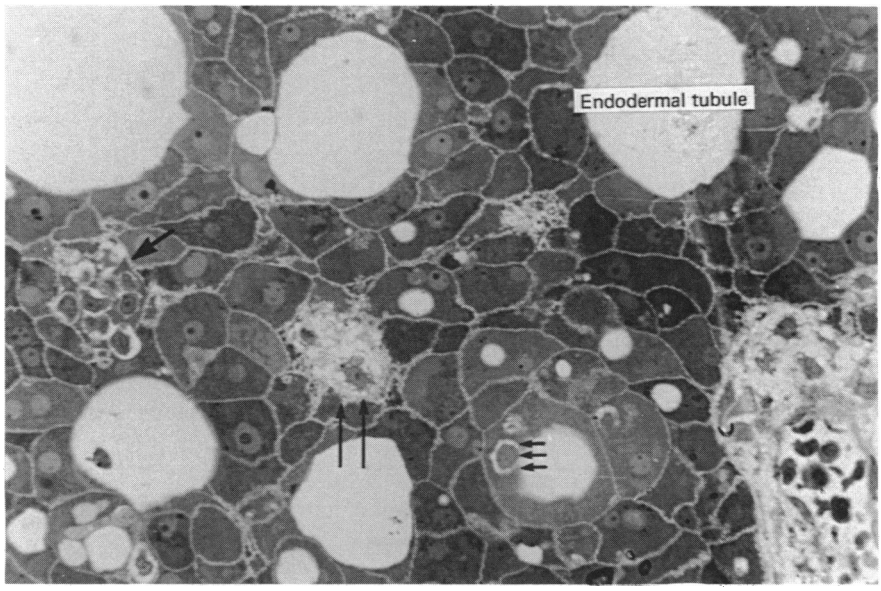

Fig. 3. In the endodermal layer, blood cells (arrow) and blood cells enclosed by mesenchyme (double arrow) are observed in the form of large and small islands. A large nucleated blood cell is observed in an intracellular tubule (triple arrow). 5th week of pregnancy. Toluidine blue. ×200.

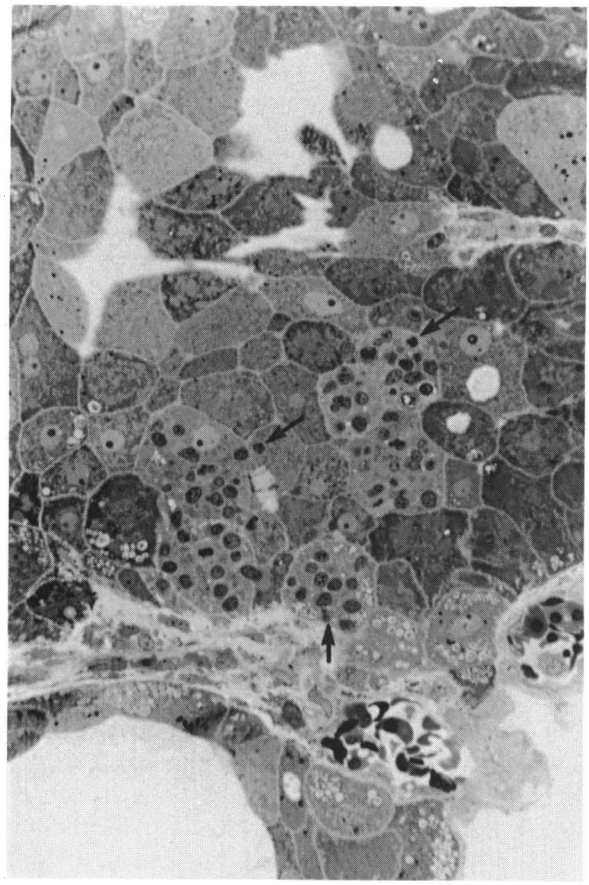

Fig. 4. Blood islands in the endodermal layer (arrows). 6th week of pregnancy. Toluidine blue. ×200.

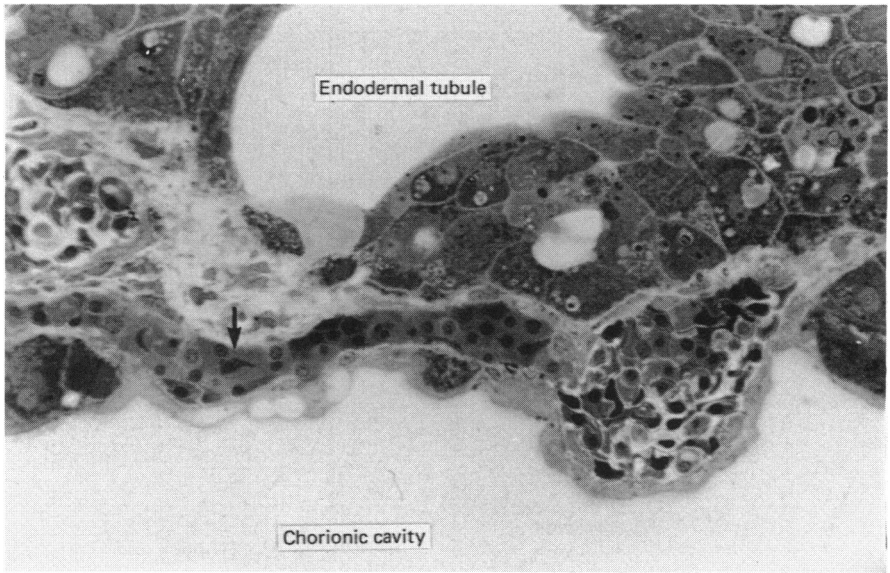

Fig. 5. Blood islands are present in the endoderm or lie between the mesenchyme and mesothelium (arrow). 7th week of pregnancy. Toluidine blue. × 300.

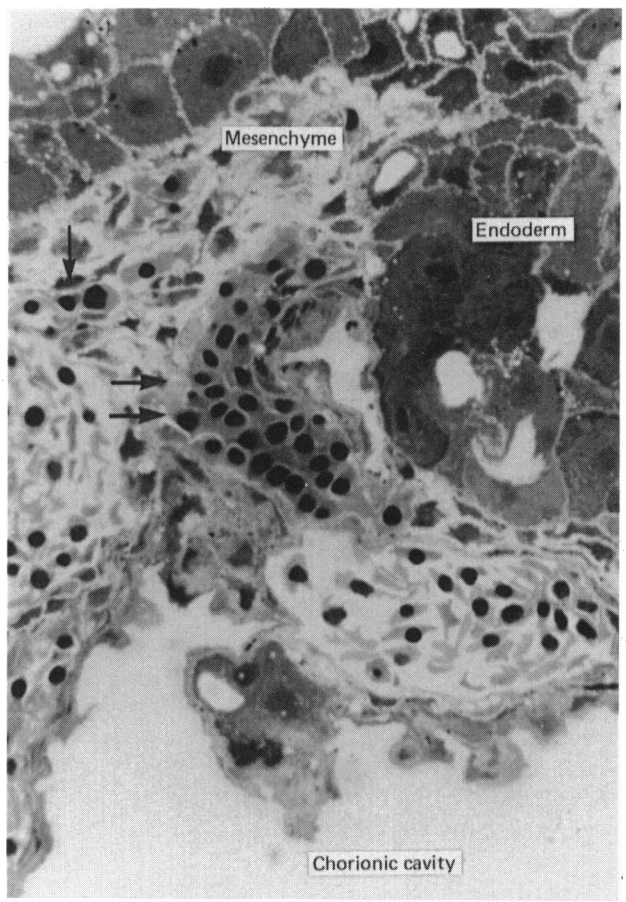

Fig. 6. A blood cell and a blood island are observed in the mesenchymal layer (arrows). 8th week of pregnancy. Toluidine blue. × 300.

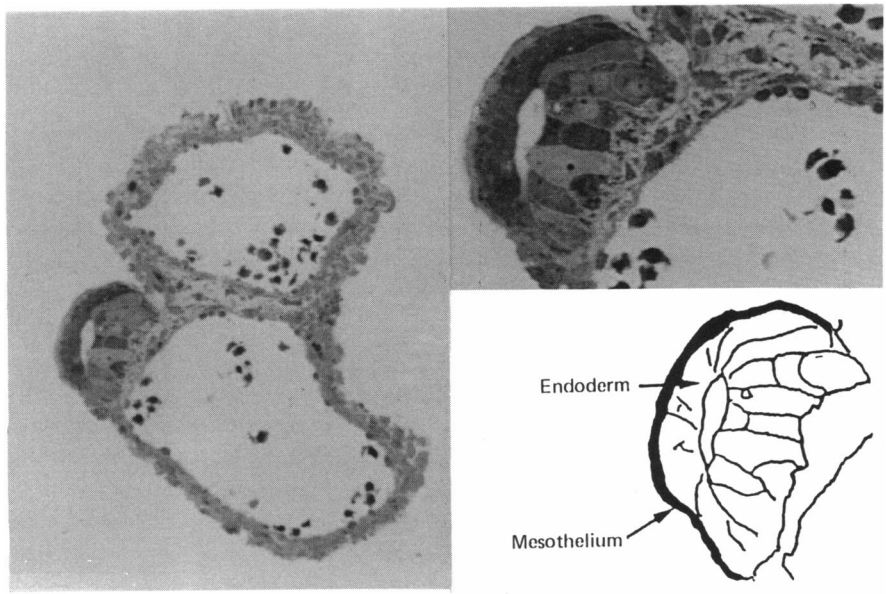

Fig. 7. A cross section of a vitelline duct (yolk duct) wall. No mesenchyme is observed between the mesothelium and endodermal tubule. Toluidine blue. ×100. Part of the micrograph is enlarged in the inset. ×200.

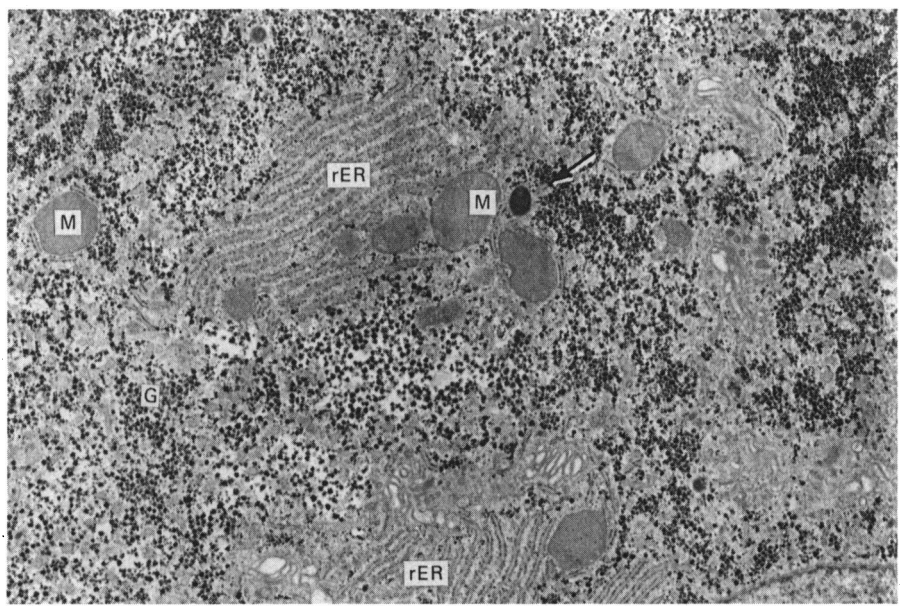

Fig. 8. The endodermal cells contain abundant glycogen (*G*), parallel cisternae of rough endoplasmic reticulum (*rER*), mitochondria (*M*) in close contact with *rER* and electron-dense granules (arrow). 6th week of pregnancy. ×8000.

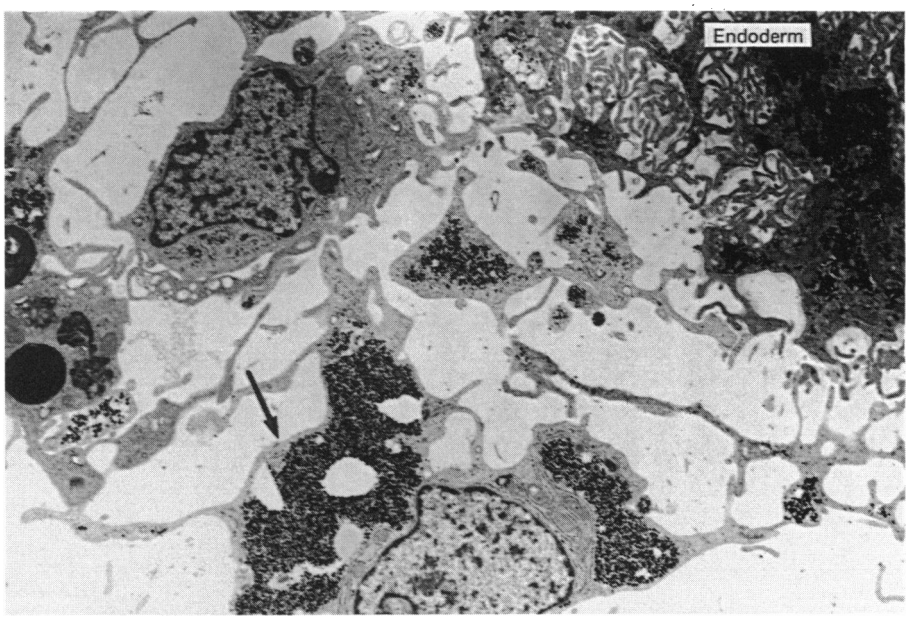

Fig. 9. The upper right of the Figure shows the mesodermal aspect of the endoderm. A mesenchymal cell, in which the organelles resemble those of an endodermal cell, is observed (arrow). 7th week of pregnancy. ×3700.

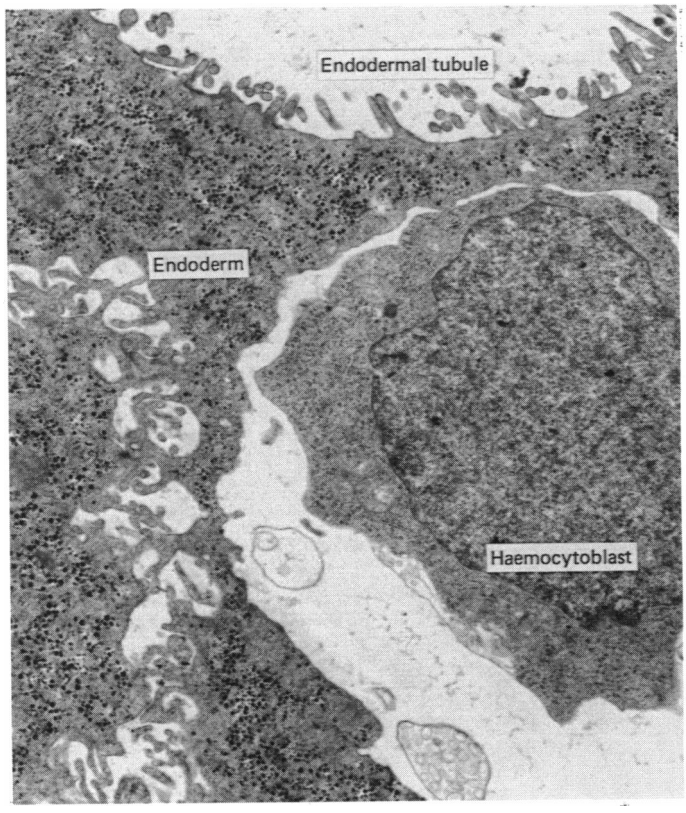

Fig. 10. A haemocytoblast is seen in an intracellular tubule. 5th week of pregnancy, ×7400.

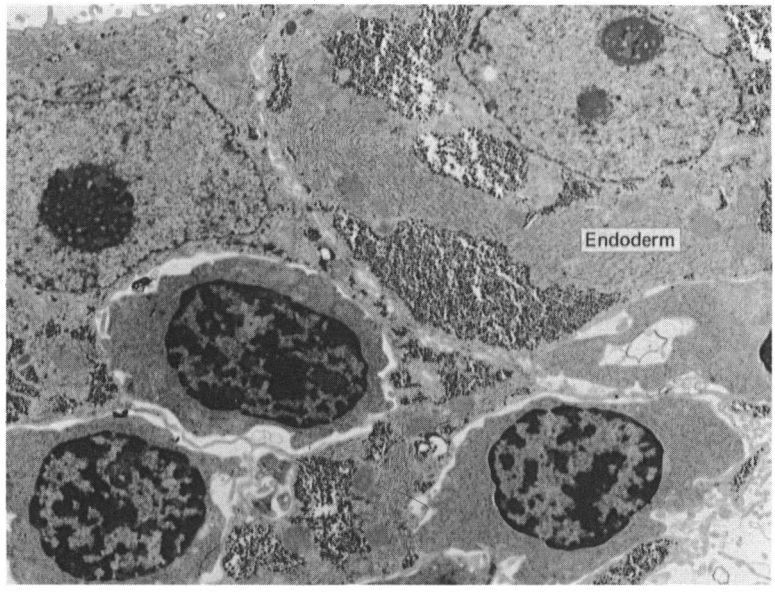

Fig. 11. Maturing blood cells in which the number of polysomes is decreased, the mitochondria are less prominent and chromatin becomes agglutinated, are seen in the endodermal layer. 6th week of pregnancy. ×4500.

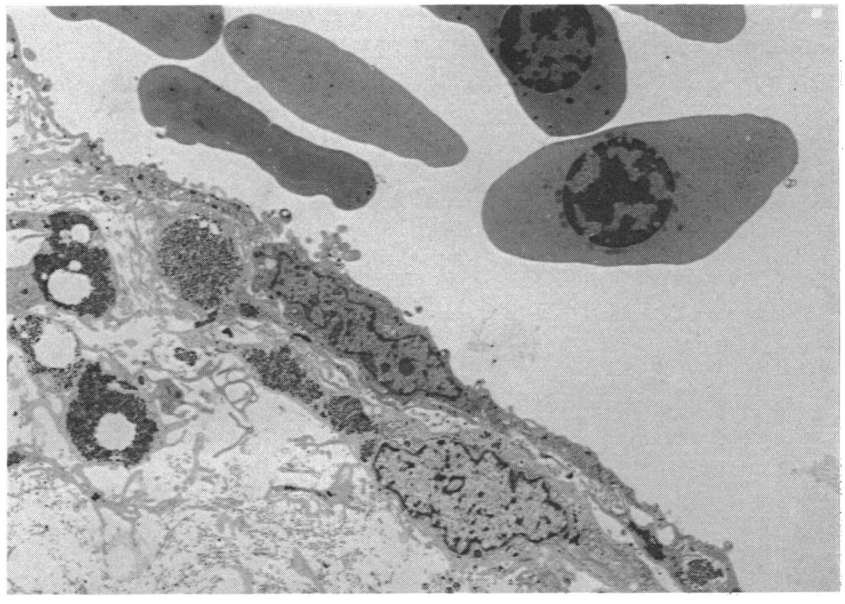

Fig. 12. The intravascular blood cells are pyknotic erythroblasts, and anucleate erythrocytes are also visible. 8th week of pregnancy. ×2600.

taining several nucleoli and abundant heterochromatin, though with less condensation of the chromatin. The cytoplasm was rich in polysomes and contained well developed mitochondria (Fig. 10). Most of the blood cells forming blood islands in the endodermal layer appeared to be more mature than haemocytoblasts, as suggested by the moderate amount of polysomes, the poor development of mitochondria and the condensation of chromatin (Fig. 11). Erythroblasts were found between the endodermal and mesenchymal layers and also in the mesenchymal tissues. The blood cells within blood vessels were pyknotic erythroblasts and erythrocytes, which were more mature than those found in intra-endodermal or intramesenchymal tissues. Electron microscopic examinations on serial sections identified some blood cells as apparently anucleate cells (Fig. 12).

DISCUSSION

The vascular system in human embryos appears in the mesoderm at about the middle of the third week of development. It is known that blood cells and blood vessels are formed several days earlier than this in extra-embryonic mesoderm such as the yolk sac walls and the chorionic membranes, and also that the yolk sac is the first haemopoietic tissue in embryos. In other words, mesenchymal tissues have been suggested as the tissues in which intravascular and extravascular haemopoiesis takes place until approximately the third week of development (Hamilton & Mossman, 1972; Langman, 1969).

In previous studies on the human yolk sac only a few cases were examined and investigations were not done at various stages of pregnancy (Fukuda, 1973). Furthermore, most of the studies were made by light microscopy on Giemsa or haematoxylin and eosin-stained preparations (Bloom & Bartelmez, 1940). In the present study, toluidine blue-stained sections prepared from Epon-embedded specimens for electron microscopy were examined. Light and electron microscopic observations on adjacent sections of the same specimens enabled us to examine the topographical relations of the cells in detail, to identify the cells morphologically and also to analyse the stage of maturation of blood cells.

The vitelline duct wall is considered to be of the same embryological origin as the yolk sac wall. Blood vessels were seen in the vitelline duct, but the mesenchyme usually seen in the yolk sac walls at all stages could not be observed between the mesothelium and the endodermal tubules. It is suggested that blood cells and blood vessels develop earlier than mesenchyme, that is, haemopoiesis might occur before the formation of mesenchyme in the yolk sac wall, and the mesenchyme in the yolk sac walls is considered to develop from other tissues at later stages, namely, after the vitelline duct becomes distinguishable from the yolk sac itself.

The yolk sac walls are approximately constant in thickness throughout pregnancy; the endoderm occupies the major part of the walls during the early stages and the mesenchyme becomes conspicuous as pregnancy proceeds. The organelles in the mesenchymal cells adjacent to the endodermal layers resemble closely those of the endodermal cells. Thus, it is suggested that the mesenchyme of the yolk sac is derived from the endoderm. Maturation of red blood cells is considered to take place extravascularly. With occasional exceptions, the blood cells in the endodermal layers were the most immature, and maturation appeared to proceed as the cells were formed in the mesenchymal layers, and further, within the blood vessels. In fact, mitotic figures in the blood cells were encountered in the endodermal layers.

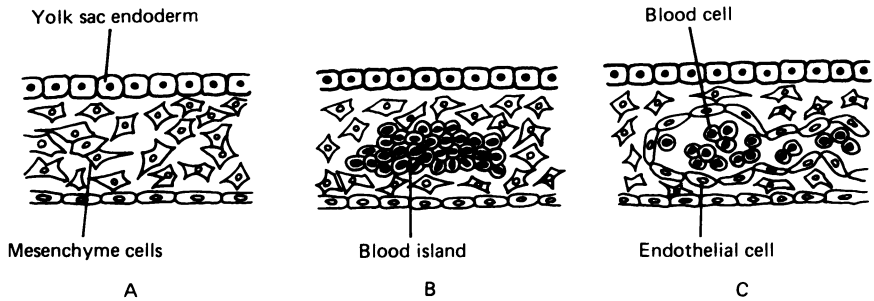

Fig. 13(A–C). Schematic diagram illustrating blood cell and blood vessel formation as usually described. (A) Undifferentiated mesenchyme. (B) Formation of angiogenic cell clusters. (C) Primitive capillary. The mesenchyme differentiates into blood cells and endothelial cells.

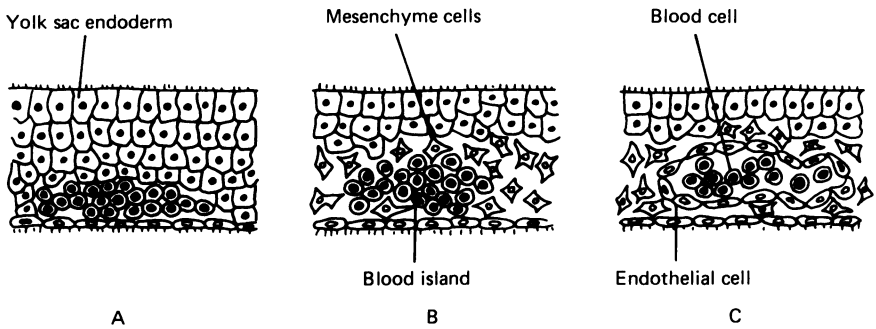

Fig. 14(A–C). Schematic diagram of the author's theory of blood cell and blood vessel formation in the yolk sac wall. (A) A blood island is formed in the endoderm. (B) The endoderm around the blood island differentiates into mesenchyme. (C) Primitive capillary. The endoderm differentiates into blood cells and mesenchyme, and the mesenchyme differentiates further into endothelial cells.

Although there may be a possibility that blood cells developed in the embryo or in the mesenchymal tissues of the yolk sacs are somehow incorporated into endoderm, it does not seem reasonable, from an embryological point of view, to assume that the blood cells migrate into endodermal tissues. The present findings suggest that haemopoiesis takes place first in the endodermal tissues, followed by migration of the blood cells into the mesenchymal tissues and ultimately into the blood vessels as maturation of the cells proceeds. The currently accepted theory, that suggests that the origin of haemopoiesis is in the mesenchyme, is expressed in Figure 13. From the present study, however, it is suggested that haemopoiesis in the human yolk sac occurs in the manner shown in Figure 14, in the endoderm, after which the mesenchyme is formed around the resulting blood cells and blood islands by differentiation from the endoderm. Finally further differentiation of vascular endothelial cells from the mesenchyme results in the formation of blood vessels.

As mentioned above, haemopoiesis in human embryos begins in the yolk sacs at about the middle of the third week of embryogenesis. Later it appears in the liver and bone marrow at the fourth to fifth week and the eighth week, respectively (Zamboni, 1965; Fukuda, 1974). At about the tenth to twelfth week, haemopoietic tissues are found distributed evenly in bone marrow throughout the body (Fukuda, 1973, 1978).

From observations of 4 human yolk sacs in the fifth week of pregnancy, Fukuda

(1973) reported the presence of erythroblastic islands in endodermal layers and assumed the presence of pluripotential stem cells originating from the endodermal cells. The present observations showed the presence of haemocytoblasts in endodermal tubules. It is postulated that the haemocytoblasts observed in the yolk sac endoderm may be stem cells.

SUMMARY

Haemopoiesis in human yolk sacs was examined using tissues obtained from a total of 27 cases in various stages of development from the fourth to eleventh week of pregnancy. In the early stages of development, the yolk sac was observed to be connected to the midgut by the vitelline duct, which became slender with later growth of the embryo. In the early stages of pregnancy, endodermal tissues were found to be a predominant component, whereas in the later stages, the mesenchymal tissues increased. The most immature blood cells and their mitotic figures were observed in the endodermal tissue. Haemopoiesis was found in endodermal tissue before mesenchymal tissue had developed. Electron microscopy revealed that maturation of the blood cells proceeded as the cells were formed in mesenchymal tissue and in blood vessels.

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