SUMMARY

Examination of yolk sac from a C3Hf and a C3H mouse with the electron microscope revealed the presence of C-type virus particles in the blood islands. Particles were observed budding from the plasma membrane of hemocytoblasts, from erythroblasts, and occasionally from reticulocytes. C-type particles were also found in similar cells in hematopoietic foci in the liver, spleen, and bone marrow of embryos, and they continued to be present in newborn C3Hf mice up to 11 days of age. Particles consistently appeared in the thymus, even in older suckling mice. A comparison is made between the presence of C-type particles in organs of embryonic, newborn, and adult C3Hf mice. C-type particles were not observed in the chorioallantoic placentas from mice that were given injections of mouse leukemia virus (Gross) or from normal noninjected mice; however, intracisternal A-type particles were present in cytotrophoblast cells from these placentas.

INTRODUCTION

In an attempt to study the natural transmission of the mouse leukemia virus (Gross) from adult C3Hf mice to their offspring, the blood-forming organs such as liver, spleen, bone marrow, and thymus of embryos from virus-injected and noninjected control mothers were examined in the electron microscope (9). An association between the presence of C-type virus particles and hematopoietic cells in these organs was observed. Although virus particles were more numerous in organs from virus-injected mothers than those from control mothers, the quantity of particles found in the embryos was similar regardless of whether they were offspring of normal, healthy mothers or virus-injected mothers. Subsequent examination of blood-forming organs of newborn mice revealed a similar association with C-type particles, which was briefly discussed in a previous report (9). Consequently, the sites of earliest hematopoiesis, the blood islands of the yolk sac, were studied and were found to contain C-type virus particles. The chorioallantoic placentas from C3Hf mice were examined in a search for morphological evidence of possible in utero transmission of C-type virus particles. Although C-type particles were not detected in placental tissue, intracisternal A-type particles were observed.

The purpose of this report is to describe the results of our electron microscopic observations of virus particles in mouse yolk sac, in the placenta, and in hematopoietic organs of newborn C3Hf mice (Table 1).

MATERIALS AND METHODS

Yolk Sac. Specimens of yolk sac, situated between the uterine wall and the amnion, were obtained from the embryos of a normal noninjected 3-month-old C3Hf mouse and those of a 7-month-old C3H mouse with mammary carcinoma. Both females were sacrificed during the latter half of gestation.

Placenta. Chorioallantoic placental tissue was obtained from 3 C3Hf mice, each sacrificed during the latter half of gestation. Two of these animals previously had received injections of passage A mouse leukemia (Gross) virus filtrate at less than 1 week of age. One virus-injected mouse was sacrificed at 2 months of age and manifested no gross or hematological evidence of leukemia. The other virus-injected mouse was leukemic when sacrificed at 3 months of age. Its spleen and cervical glands were enlarged; thymic and mesenteric tumors were also present. Placental tissue from a 3-month-old normal, noninjected C3Hf mouse was also studied.

Newborn Mice. Thymus, spleen, liver, and bone marrow from normal noninjected C3Hf mice were examined. The mice ranged in age from 1.5 to 18 days.

METHODS. Placentas, yolk sacs, and tissues from newborn mice were processed and embedded as previously described (11). Thin sections were cut on a Porter-Blum microtome with a diamond knife; the sections were then coated with carbon and stained with uranyl acetate, followed by either lead citrate (32) or lead hydroxide. They were examined in a Philips Model 300 or RCA Model 3G electron microscope.

RESULTS

Yolk Sac. Sections of yolk sac examined in the electron microscope (Fig. 1) contained blood islands made up of...
hemocytoblasts, erythroblasts, reticulocytes, megakaryocytes, and macrophages. The hemocytoblasts were large, almost spherical cells (Fig. 3). Their nuclei contained 1 or more nucleoli and fine, homogenously distributed chromatin with few areas of marginal clumping. The nuclear membrane was indented and interrupted by numerous nuclear pores. Some strands of rough-surfaced endoplasmic reticulum, a few mitochondria, a Golgi zone, centrioles, and abundant ribosomes were present in the cytoplasm.

Cellular changes accompanying the differentiation from hemocytoblast to erythroblast and the maturation of erythroblast included a decrease in cell size, greater sphericity of both cell and nucleus, a larger nucleo-cytoplasmic ratio, an increase in density of cytoplasm and nucleus, and a diminution in the number of cell organelles.

In embryonic bone marrow, particles appeared in all 3 specimens examined. In bone marrow from the newborn mice, particles were observed in 4 of 8 specimens, all from mice less than 12 days of age. Particles were not found in the livers from older offspring and adult mice.

In the thymus, particles were formed from the plasma membrane of lymphoid cells and from the vacuolar membrane of epithelial cells. In liver, spleen, and bone marrow, particle formation was observed only in the cells in hematopoietic foci. C-type particles are shown (arrows) budding from an erythroblast in liver (Fig. 7) and a hemocytoblast in spleen (Fig. 8) from a 9-day-old mouse and from a lymphoid cell in the thymus of a 4.5-day-old mouse (Fig. 9).

**DISCUSSION**

Results of this study and previous experiments (9) demonstrated the formation of C-type virus particles by budding from the plasma membrane of hemocytoblasts, of erythroblasts, and occasionally from reticulocytes in yolk sac, spleen, liver, and bone marrow of embryos and newborn C3Hf mice. Since virus particles were not found in other cells of these tissues, these findings suggest a relationship between C-type virus particle formation and hematopoiesis. Additional evidence may be found in a study (33) in which C-type particles were described in the liver, spleen, and thymus, but not in muscle of embryonic and newborn mice.

The ontogeny and interrelationships of the blood-forming organs in the embryo, newborn, and adult mouse may account, in part, for the results obtained in this study. The
embryonic and early neonatal periods are times of intense erythropoietic activity in the mouse. From the 7th or 8th to the 11th day of gestation, the blood islands of the yolk sac are reported to be the only source of erythrocytes in the mouse embryo (25). From the 12th to the 16th day of gestation, the liver is cited as the major erythropoietic organ and continues as an important source of erythrocytes up to birth. It may contain foci of erythropoietic activity for 1 week after birth (25) or slightly longer, as demonstrated in this experiment. Before birth, on the 15th and 16th days of gestation, erythropoiesis is reported to begin in the spleen and bone marrow, respectively (25).

Studies have shown that the mouse yolk sac is the first organ to form circulating stem cells capable of populating the future hematopoietic organs of the embryo (22). The requirement of embryo liver for such colonization by yolk sac stem cells in the formation of hematopoietic foci was reported (22). In addition, transplanted yolk sac stem cells were shown to be capable of repopulating the lymphoid tissue of thymus and peripheral lymph nodes and the myeloid tissue of spleen and bone marrow of lethally irradiated hosts (22).

The hematopoietic potential of stem cells in the 12-day-old mouse embryo liver was demonstrated when these cells proliferated in the thymus and bone marrow of lethally irradiated adult hosts (30). In other experiments, i.v. injections of cell suspensions from fetal liver or fetal blood administered to lethally irradiated mice were responsible for the recovery of hematopoietic tissue (1, 2, 19). It was proposed that the circulating hematopoietic stem cells in fetal blood came from the liver and colonized the developing bone marrow, which, later, in the adult, is the main site of hematopoiesis (1). According to other studies, hematopoiesis in the thymus is dependent on stem cells from the yolk sac and embryo liver invading the thymic rudiment between 10 and 11 days of gestation via the blood stream (23, 24).

There is evidence that hematopoietic tissue from bone marrow of the adult mouse contains cells that are self-sustaining and that are probably stem cells (6). It has been suggested that the stem cells of thymic lymphocytes of the adult mouse are derived from the bone marrow (12, 30, 31). Results of studies described in a review of this subject (21) indicate that adult bone marrow hematopoietic cells are capable of continued proliferation, independent of circulating stem cells; whereas hematopoietic tissue in adult spleen, lymph node, and thymus are continually replaced by stem cells from the peripheral circulation.

The existence of hematopoietic cells that migrate via the circulation between different organs in the embryo, newborn, and adult mouse may explain, to some degree, the organ distribution of C-type particles at various ages in the mice examined in this experiment. If stem cells that form C-type particles in blood islands of the yolk sac are the origin of hematopoietic cells in embryo liver, bone marrow, thymus, and spleen, this could account for the presence of such particles in these organs. In any case, the production of C-type virus particles seems to be associated with hematopoiesis. C-type particles were observed in hematopoietic zones in embryonic spleen, liver, and bone marrow. However, they were not detected in liver of mice beyond 9 days of age, when hematopoietic foci had almost disappeared, or in spleen of mice older than 11 days, when hematopoietic foci were decreased in number. During the fetal and early neonatal growth period, there is a greater demand upon the bone marrow for erythrocyte production than in the adult (35). In the electron microscope, more aggregates of erythropoietic cells were found in bone marrow from embryo and younger neonates than in adults. As compared to embryos and younger newborn mice, particles were observed less frequently in the bone marrow of mice over 7 days of age and only rarely in the adult. Although the bone marrow and, to a much lesser extent, the spleen retain their erythropoietic function in the adult mouse, observation of C-type particles may be contingent upon the number of erythropoietic cells present. Erythropoietic cells are estimated to be 2.2% of the adult mouse spleen (Ref. 20, p. 13) and 27% of the bone marrow (Ref. 20, p. 22). Since the sections prepared for the electron microscope are minute tissue samples, the likelihood of viewing erythropoietic cells present in limited numbers is reduced. Even in thin sections of yolk sac, embryo liver, spleen, and bone marrow containing easily localized erythropoietic zones, the detection of C-type particles sometimes required examination of many hematopoietic foci, since these particles were not found in every such area examined. It is possible that variation in the quantity of C-type particles present in embryo and adult mice may reflect differences in the proliferative activity between embryo and adult erythropoietic cells.

The dependence of the thymus upon the other blood-forming organs (the yolk sac and liver in the embryo and probably the bone marrow in the adult) may explain the presence of particles in the thymus, since the source of the thymocytes would then be particle-containing stem cells of yolk sac, embryo liver, and adult bone marrow. However, the significance of the observation of C-type particles in thymic epithelial cells is unclear at this time.

The C-type virus particles observed in this experiment presumably represent a latent leukemogenic virus. Mice of many laboratory strains, including those of low-leukemic inbred lines such as C3Hf and C3H, carry latent, potentially leukemogenic viruses that may be activated by various endogenous or exogenous factors (13-15). These viruses are transmitted vertically from one generation to another, presumably through the germ cells (13, 15).

Preliminary examination of C3Hf mouse placenta in our laboratory did not reveal the presence of C-type virus particles. However, in other laboratories, C-type particles were found in placentas from several strains of mice (27, 28). Recently, a small number of similar particles was described in localized areas of rat placenta (16). The appearance of C-type particles has been extensively reported in the placenta of primates (7, 8, 17, 18, 26, 34).

The paucity of C-type particles in mouse and rat placentas, as compared to the relative facility with which these particles can be located in primate placenta, is of interest. Differences between the mouse and rat and the primate, such as the number of trophoblastic layers in the placenta, the length of gestation period, and the presence of a yolk sac placenta, perhaps contribute to the variation observed.
in C-type particle morphological expression.

Intracisternal A-type particles were previously reported in placentas of several strains of mice (29), and our study corroborates this finding. However, the presence of intracisternal A-type particles in mouse placenta is not surprising, since these particles are ubiquitous in the mouse and have already been described in a large variety of normal tissues (10, 11). In addition, intracisternal A-type particles were reported in mouse egg cylinders and in 2- to 5-celled embryos to the blastocyst (3, 5), and in dictyate oocytes (primary oocytes) immediately after release from the ovarian follicle (4). The biological significance of these A-type particles has not yet been determined.

In conclusion, the evidence presented in this study indicates an association between C-type virus particle production and hematopoesis in C3Hf mice. This relationship is more apparent during periods of intense erythropoietic activity in embryonic and early postnatal development.

ACKNOWLEDGMENTS

The valuable assistance of Lorraine A. Moore of the Veterans Administration Hospital, Bronx, N. Y., and Janet Becker of Hoffmann-La Roche, Inc., Nutley, N. J., is gratefully acknowledged by the authors.

REFERENCES


Fig. 1. Section through blood island of yolk sac showing hemocytoblast (H), erythroblast (E), reticuloocyte (R), and macrophage (M). x 15,600.
Fig. 2. An erythroblast from blood island of yolk sac x 30,000. 3a, higher magnification of outlined area of Fig. 2, demonstrating a budding C-type virus particle (arrow). x 150,000.
Fig. 3. A hemocytoblast from blood island of yolk sac. x 26,000. 3a, higher magnification of the outlined area showing a budding (arrow, lower right) and an immature (arrow, upper left) C-type particle. x 100,000.
Fig. 4. A double intracisternal A-type particle (arrow) budding from the endoplasmic reticulum of a connective tissue cell in yolk sac. x 140,000.
Fig. 5. Section through trophoblast of placenta showing 3 cell layers (1, 2, 3) and an endothelial cell (E) of a fetal capillary. x 22,000. 5a, enlargement of the outlined area showing an intracisternal A-type particle (arrow) in the endoplasmic reticulum of a cytotrophoblast cell. x 130,000.
Fig. 6. Part of a cell from the cytotrophoblast layer of the placenta. Budding and free intracisternal A-type particles (arrow) appear within the endoplasmic reticulum. x 150,000.
Fig. 7. Section through an erythroblast from liver of a 9-day-old mouse. A C-type virus particle (arrow) budding from the erythroblast is shown. x 75,000.
Fig. 8. A C-type virus particle (arrow) budding from a hemocytoblast in spleen from a 9-day-old mouse. x 70,000.
Fig. 9. Part of a lymphoid cell from thymus of a 4.5-day-old mouse. A C-type virus particle (arrow) budding from the plasma membrane. x 44,000.
Virus Particles in Yolk Sac, Placenta, and Hematopoietic Organs

MAY 1976

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association of toxicology. Persons not members of such societies may present papers at this meeting if they are sponsored by a member of a recognized group. Abstracts should be submitted to the secretary no later than October 1, 1976.

Program, accommodations, and registration information for the meeting will be sent to all those who request it. All requests for information should be directed to Dr. Robert G. Burford, Secretary, ICT, c/o G. D. Searle & Co. of Canada, Ltd., 400 Iroquois Shore Road, Oakville, Ontario, Canada, L6H 1M5.

**BREAST CANCER: A REPORT TO THE PROFESSION, 1976**

A conference on “Breast Cancer: A Report to the Profession, 1976” will be held on November 22 and 23, 1976, at the Washington Hilton Hotel, Washington, D. C. The conference is sponsored by the White House and the National Cancer Institute and is supported with funds resulting from the sale of President Ford’s Inaugural Medals and Plates. The objective of the program is to report and discuss major problems concerning biology, epidemiology, diagnosis, and treatment of breast cancer today. The conference is open to all members of the medical and health profession and to the public. Advanced registration is requested; there is no registration fee. For further information write to Ms. Sally Simpson, Breast Cancer Conference, 1501 Wilson Boulevard, Sixth Floor, Arlington, Virginia 22209.

**Erratum**

The following change should be made in the article, entitled “Electron Microscopic Study of Virus Particles in Yolk Sac and Placenta and in Hematopoietic Organs of Newborn C3Hf Mice,” by Dorothy Feldman, Ludwik Gross, and Richard L. Swarm, published in the May 1976 issue of the Journal. The first sentence of the “Materials and Methods” section should read: “Specimens of yolk sac, situated between the uterine wall and the amnion, were obtained from the embryo of a normal noninjected 3-month-old C3Hf mouse and that of a 7-month-old C3H mouse with mammary carcinoma.”
Electron Microscopic Study of Virus Particles in Yolk Sac and Placenta and in Hematopoietic Organs of Newborn C3Hf Mice

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