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# Teratoma Induction in Mice and Rats in Relation to the Age of the Visceral Yolk Sac*

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**Abstract**—*The morphological and biological characteristics of yolk sac-derived mouse teratomas are compared to those induced in rats. In both species, similar well differentiated adult tissues were recorded. The differential potential of the yolk sac was identical between the different mouse and rat strains tested but was very much dependent upon the age of the yolk sac at the time of operation. Killing of the primary germ cells by Busulphan treatment did not influence the development of these teratomas.*

## INTRODUCTION

IN THE mouse the development of benign teratomas and malignant teratocarcinomas is very well documented. These tumors either appear spontaneously in the gonads or are induced by the ectopic transplantation of genital ridges, eggs or young embryos [1–4]. After ectopic implantation of embryonal tissues the mouse develops, depending upon the strain, either a teratocarcinoma (C3H, 129 strain), or a teratoma (CB7B1) [5]. In all cases these tumors are believed to be of germ cell or embryonal cell origin. Since in our model system in the rat the teratomas derive from yolk sac [6, 7] and are not of germ cell origin [8] we wanted to verify whether in different mouse strains similar teratomas could be obtained and whether a germ cell origin could be excluded. In order to exclude a germ cell origin of yolk sac-derived mouse teratomas, we treated pregnant animals with busulphan. This drug is known to destroy the germ cells during their migratory phase from the yolk sac to the genital ridges [9–11]. Furthermore, we investigated the possibility that in the mouse and in the rat the induction of yolk sac-derived teratomas may not only be related to the strain of inbred animals used but also to the age of the yolk sac at the time of operation.

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## MATERIALS AND METHODS

Mice of the inbred strains C3H and C57B1 and rats of the inbred strains R and BN were used.

In 12 C3H female mice copulated with C3H males and 10 C57B1 females mated with C57B1 males fetectomy was performed at day 11 of pregnancy and the yolk sac was pulled outside the uterine horns, as previously described in rats [6]. Moreover, 2 groups of C3H females copulated with C3H males were treated with busulphan (Wellcome and Co., London, England) at day 9 of pregnancy. The drug was injected intraperitoneally (3 or 5 mg/100 g body weight) suspended on 0.2 ml arachis oil, the control pregnant mice receiving the arachis oil only. At day 11 of pregnancy the fetectomy was performed as usual. the fetuses being removed from one uterine horn only, the contralateral horn was left intact. The newborn mice from the treated and control mothers were killed at birth and the gonads were taken out and fixed for histological studies.

In 12 R females rats copulated with R males, 10 BN females mated with BN males fetectomy was performed at day 12 of pregnancy.

Two groups of pregnant C3H female mice copulated with C3H males were fetectomized at day 13 or 15 after mating. Three groups of R female rats copulated with R males were fetectomized at day 10, 15 or 17 of pregnancy. Each group contained 12 animals.

The method of operation was the same, except that at day 10 the fetectomy had to be performed under a dissection microscope as the very small embryos were attached to the visceral yolk sac but not surrounded by it.

From each group of the intrastrain pregnant R rats and C3H mice fetectomized at different days, 2 females were killed 2 days after operation and the sites of the uterus near the incision were fixed in glutaraldehyde, postfixed in osmium and embedded in Spurr. From serially cut thick sections the areas containing visceral yolk sac outside the uterus was chosen and thin sections made from them. These were strained with uranyl acetate and lead hydroxide and examined under the electron microscope (Zeiss).

The remaining mice and rats from each group were killed 21 days after operation. The uteri were fixed in formol, and paraffin blocks containing tumors were cut serially. The slides were stained with hematoxylin and eosin.

## RESULTS

1. *Morphology of yolk sac* 2 days *after fetectomy*

*In the mouse.* The youngest yolk sac fixed 2 days after operation (fetectomy at day 11) was, when studied in thick sections, similar to that found *in utero* at the day of fetectomy, except that there were fewer nucleated erythroblasts in the vessels (Fig. 1). The epithelial cells lay on a thin basement membrane, which separated them from numerous mesenchymal cells. Between these cells vessels containing erythrocytes were seen. A very thin serosal basement membrane separated this mesenchymal layer from elongated mesothelial cells.

At the ultrastructural level the endodermal cells were partially separated by spaces at the points of interconnection by desmosomes, they possessed irregular microvilli which were not very long. In the cytoplasm distinct mitochondria, abundant endoplasmic reticulum, poorly developed Golgi apparatus, phagosomes, lipid droplets, small vacuoles and free ribosomes were observed. Nuclei were irregular, rich in chromatin and lay in the basal part of the cells (Fig. 2). A thin basement membrane containing some collagen fibrils separated this endodermal epithelium from mesenchymal cells. These were characterized by the presence of dilated ergastoplasms, many free ribosomes and an irregular nucleus (Fig. 3). Endothelial cells were poorly differentiated with many ribosomes and occasional cytoplasmic organelles. On the serosal basement membrane, together with some young collagen fibrils, lay elongated mesothelial cells. Their cytoplasm was also poorly differentiated, with numerous free ribosomes. The elongated nuclei rich in chromatin, were in the middle of the cell.

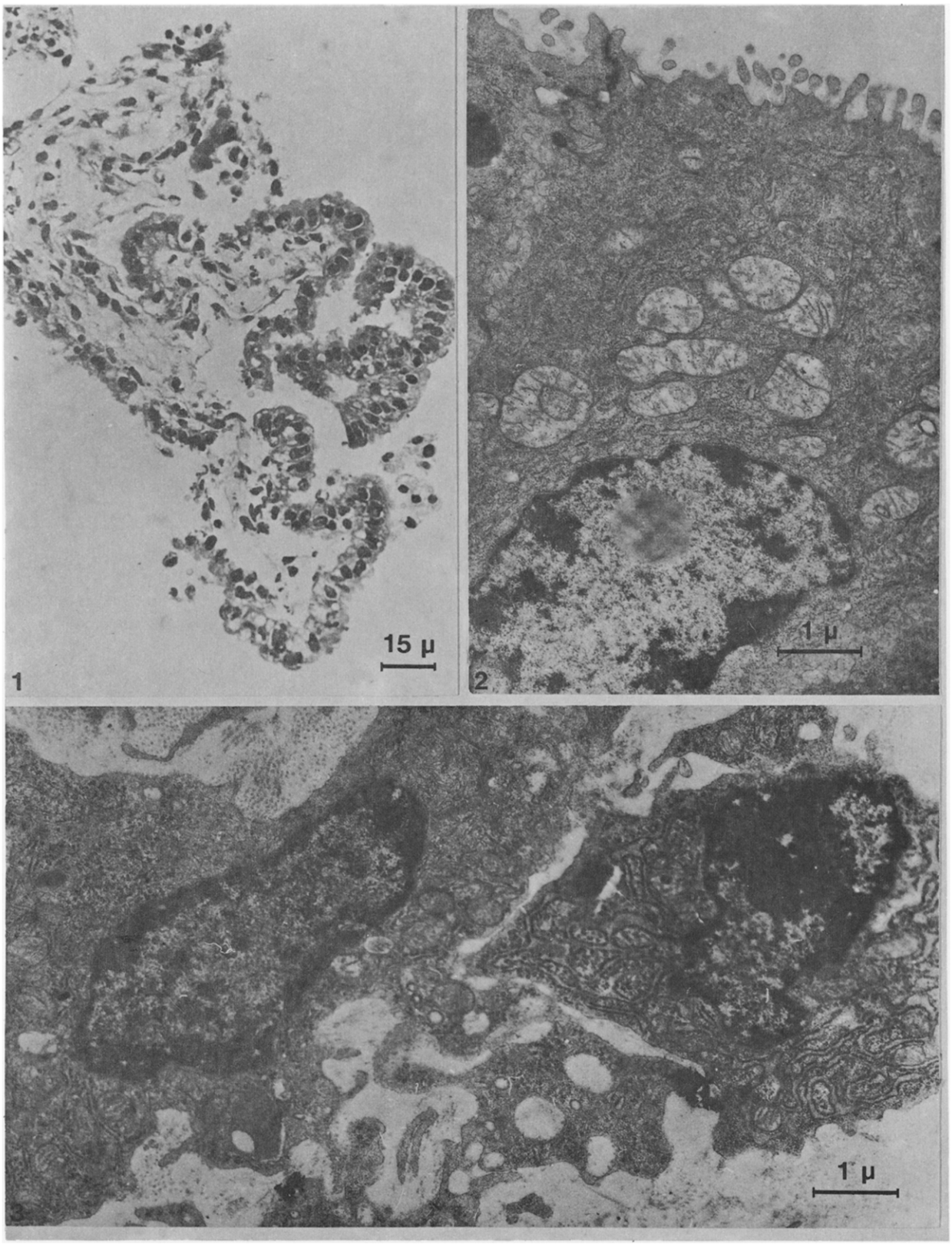
The histology of the visceral yolk sac pulled outside the uterus at the 13th day of pregnancy and fixed 2 days later, differed from that described previously. The epithelial cells seemed flat instead of columnar, the mesenchymal cells were less numerous and the basement membranes were thicker.

In the electron microscope the endodermal cells showed shorter and less regular microvilli, numerous phagosomes, lipid droplets, dilated endoplasmic reticulum and only a few free ribosomes (Fig. 4). Nucleoli were rarely seen in the nuclei. The visceral basement membrane was thicker than at the 11th day and contained more collagen fibrils, which were also abundant between mesenchymal cells. These latter cells were few in number, and their cytoplasm contained numerous cisternae filled with a homogeneous substance. The serosal membrane was very thick, mesothelial cells flat and long with rare short microvilli (Fig. 5).

Histologically the 2-day older mouse yolk sac (15th day of pregnancy) showed only necrotic cells and a very thick basement membrane (Fig. 6).

On the ultrastructural level the necrotic aspect of the endodermal cells was characterized by a cytoplasm filled with vacuoles and membranes; no other structures were seen (Fig. 7). The other cell layers of the visceral yolk sac were replaced by a very thick basement membrane with collagen fibrils embedding single necrotic mesenchymal cells.

*In the rat.* Histological and ultrastructural examination of visceral yolk sac of rats left outside the uterus for 2 days after fetectomy showed that the morphology of this membrane is very similar to that in the earlier stages of pregnancy in mice. Indeed, the 12-day old yolk sac of rats had the same structure as that at 11 days in mice, 15-day old membrane of rats conformed to that at 13 days in mice, but 17-day old rat yolk sac was not identical to that at 15 days in mice. In 17-day old yolk sac similar cells to those described in 15-day old rat yolk sac were also observed between necrotic endodermal cells. These cells had short and irregular micro-

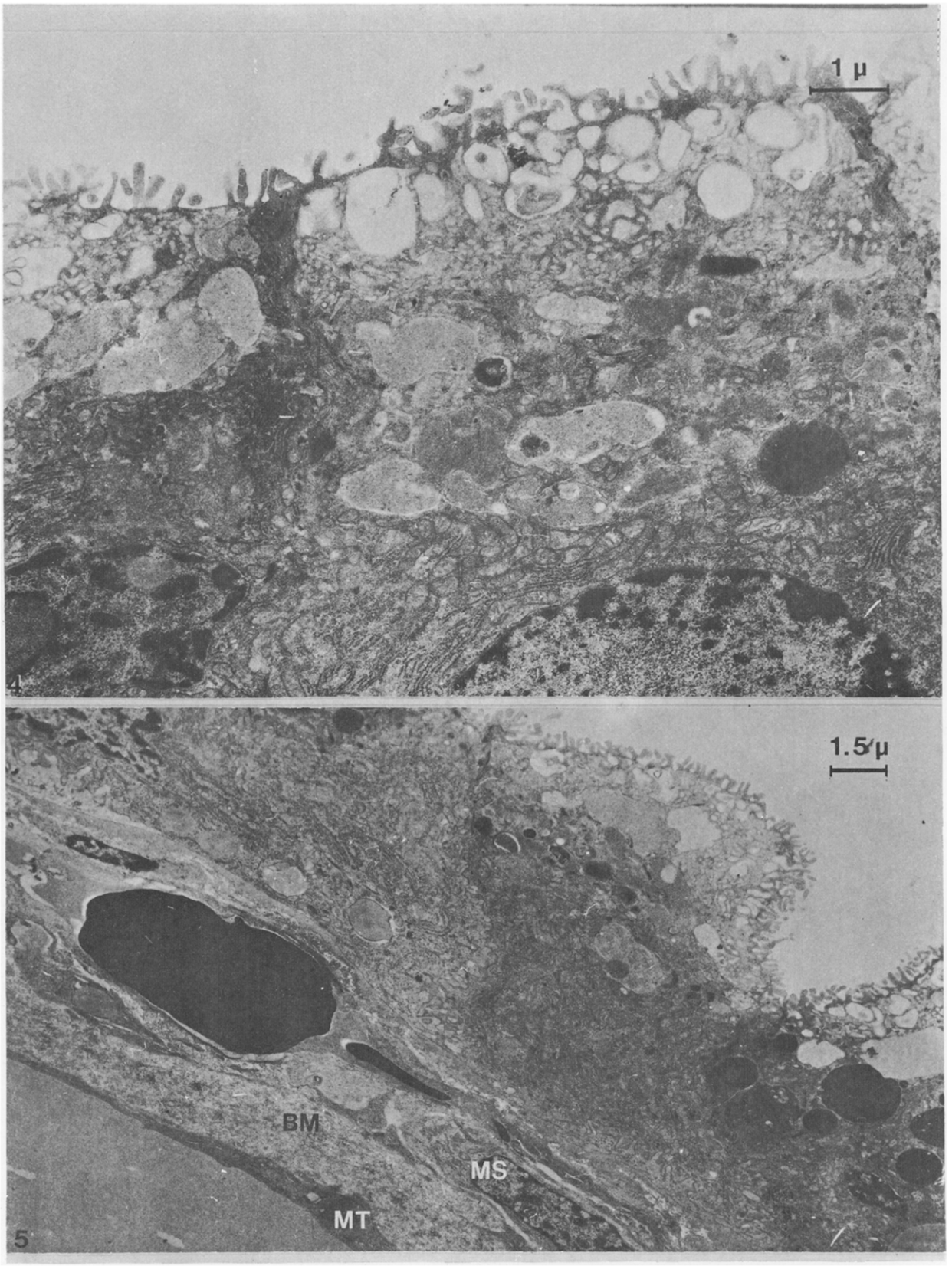



*Figs.* 1–3. *Morphology of* 11-day *old mouse visceral yolk sac left outside the uterus for* 2 days *after fectectomy.*

*Fig.* 1. *Structure similar to* 11-day *old yolk sac* in situ. *H and E.*

*Fig.* 2. *Ultrastructure of an endodermal cell with irregular microvilli and all organellae.*

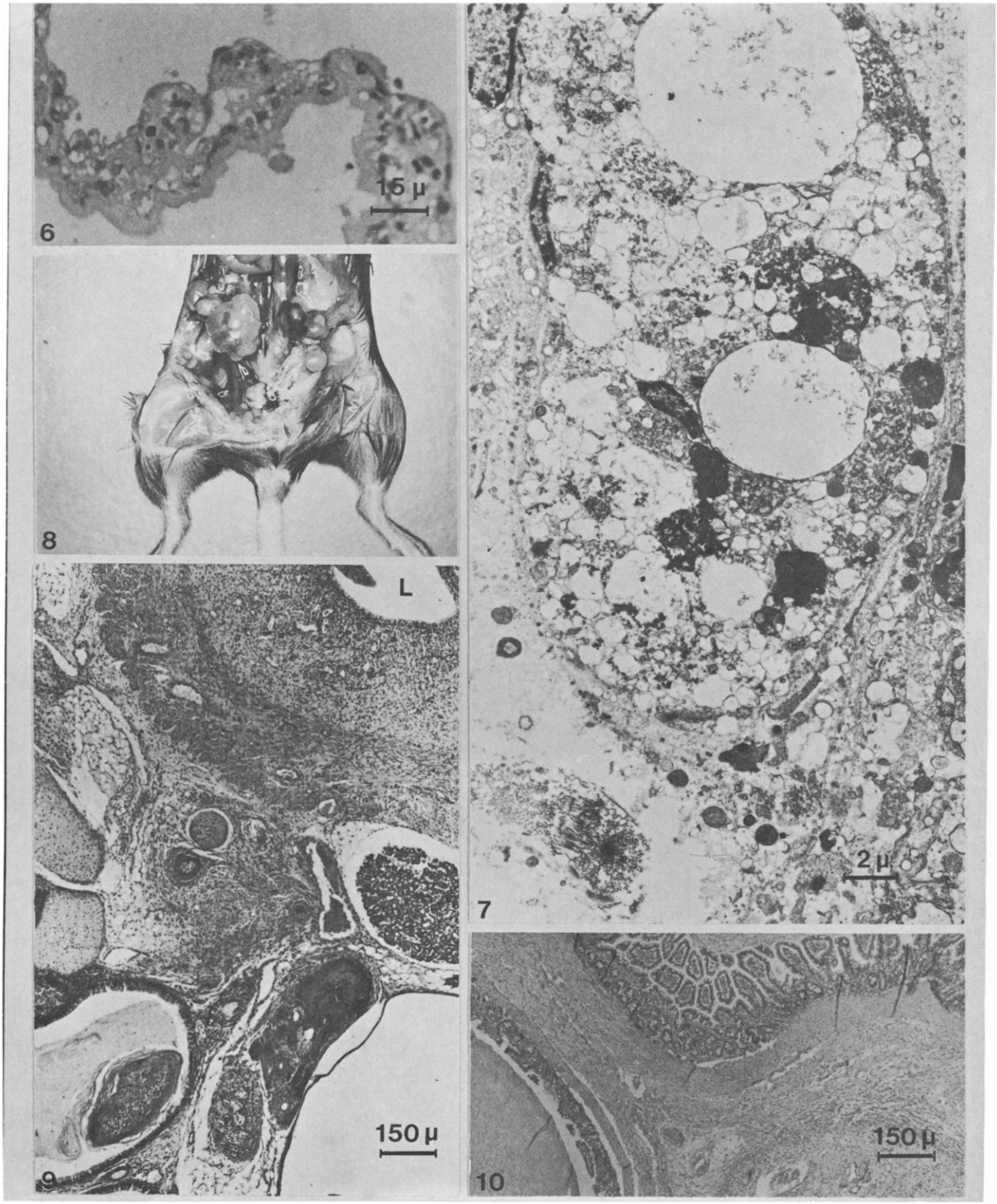
*Fig.* 3. *Mesenchymal cell with dilated ergastoplasm, free ribosomes and irregular nucleus.*




*Figs. 4 and 5. Ultrastructure of* 13-day *old mouse visceral yolk sac left outside the uterus for* 2 days *after fetectomy.*

*Fig. 4. Endodermal cell with irregular microvilli, vacuoles and phagosomes.*

*Fig. 5. Thick basement membranes with collagen (BM), a mesenchymal cell with cysterns (MS) and a flat mesothelial cell (MT).*




*Figs.* 6 *and* 7. *Structure of* 15-day *old mouse yolk sac left outside the uterus for* 2 days *after fetectomy.*

*Fig.* 6. *Necrotic endodermal cells and thick basement membrane H. and E.*

*Fig.* 7. *Ultrastructure of necrotic endodermal cells.*

*Fig.* 8. *Teratomas developed in mouse* 3 weeks *after fetectomy.*

*Fig.* 9. *Histological section of teratoma developed from* 11-day *old yolk sac. Various weel-differentiated tissues are seen. L=uterus lumen. H and E.*

*Fig.* 10. *Histological section of teratoma developed from* 13-day *old yolk sac. Endodermal cysts are seen. H. and E.*

villi, their cytoplasm contained dilated endoplasmic reticulum, lipid droplets; phagosomes and a few ribosomes. The 10-day old yolk sac in rats was less differentiated than the older ones we described. Many mitotic figures were seen in the endoderm. The epithelial basement membrane was thin but visible. Endothelial cells formed capillaries. Occasionally the histological examination of the uterine horns taken out 2 days after fetectomy which was performed at day 10 of pregnancy, showed the presence of small fragments of embryo outside the uterus. When the fetectomies were performed at a later stage, these embryonic structures were never observed in serial sections of uterine horns which had yolk sac left outside.

2. *Morphology of gonads of newborn mice from busulphan-treated mothers*

The testes and ovaries of newborn mice from mothers treated with arachis oil only were rich in large, round germ cells. In the gonads of newborns from mothers treated with busulphan in doses of 3 mg/100 g body weight, the germ cells were nearly absent compared to oil-treated control animals. The testes and ovaries of these newborns from busulphan-treated mothers contained only rare degenerated germ cells. In the pregnant mice treated with 5 mg busulphan/100 g body weight no living fetuses were born, most probably due to the toxic effect of the drug.

3. *Teratomas*

*In mice.* Twenty-one days after operation, all female C3H mice mated with C3H males, treated with busulphan or not and all C57Bl females copulated with C57Bl males and fetectomized at day 11 of pregnancy, developed tumors.

The incidence of teratomas in different strain combinations is shown in Table 1. The numbers of tumors in both strains of mice varied from 1 to 4 per uterine horn, the size varied from 0.2 to 2 cm in diameter. They were all well encapsulated, had a smooth surface and remained attached to the uterine wall (Fig. 8). After incision some of them displayed several cysts containing a yellow to reddish mucinous fluid.

Histological examination of these tumors revealed endodermal cysts, gut formation, pancreas, epidermal cysts with skin derivatives such as hairs and sebaceous glands, cartilage, bone and bone marrow (Fig. 9). The bone marrow contained different cell types such as erythroblasts, myeloblasts, megakariocytes, plasma cells and reticulum cells. We also observed skeletal muscle and, occasionally, nervous tissue. Less frequently the tumors also contained bronchiolar epithelium, lymphoid tissue and in one case formation of the liver. These findings are summarized in Table 2. There was no difference between C3H and C57Bl mice in the variety of tissues present in the tumors.

*Table 1. Incidence of yolk sac-derived teratomas in different strain combinations in mice and rats*

| No. of animals | Strain | No. of fetectomies* | No. of tumors |
|---|---|---|---|
| 10 | C3H × C3H | 79 | 37 |
| 10 | C57Bl × C57Bl | 74 | 35 |
| 10 | R × R | 55 | 26 |
| 10 | BN × BN | 52 | 23 |

*All fetectomies were performed at day 11 of pregnancy in mice and at day 12 in rats.

*Table 2. Appearance of various tissues in teratomas derived from yolk sac of different age*

| Tissues observed | Mice* Day of fetectomy | | | Rats Day of fetectomy | | | |
|---|---|---|---|---|---|---|---|
| | 11 | 13 | 15 | 10 | 12 | 15 | 17 |
| Endodermal cyst | 37 | 32 | 1 | 16 | 14 | 20 | 14 |
| Gut | 31 | 28 | – | 12 | 11 | 15 | 10 |
| Pancreas | 12 | 8 | – | 7 | 8 | 5 | – |
| Liver | 1 | – | – | – | – | – | – |
| Squamous epithelium | 17 | 5 | – | 14 | 15 | 2 | – |
| Skin derivatives | 11 | 1 | – | 12 | 13 | – | – |
| Neural tissue | 3 | – | – | 2 | 3 | – | – |
| Chondroid tissue | 12 | 1 | – | 13 | 14 | 3 | 1 |
| Bone | 18 | 1 | – | 12 | 12 | 2 | 1 |
| Bone marrow | 17 | 1 | – | 12 | 11 | – | – |
| Muscular tissue | 9 | – | – | 11 | 10 | – | – |
| Lymphoid tissue | 10 | – | – | 7 | 5 | – | – |
| Total number of tumors | 37 | 32 | 1 | 24 | 26 | 20 | 14 |

*Each group of mice and rats fetectomized at different days of pregnancy consisted of 10 animals.

The macroscopical and microscopical appearance of tumors developed in C3H mice treated with busulphan during pregnancy, was identical to those developed in the control group. Even in the animals which were treated with a dose (5 mg/100 g body weight) lethal for the fetuses, teratomas were observed in the fetectomized uterine horns (Table 3).

*In rats.* In rats operated on at day 12 of pregnancy the results were similar to those obtained in mice fetectomized at day 11. Yolk sacs gave rise to tumors in all rats of the syngeneic crosses (R × R, BN × BN).

*Table 3. Incidence of yolk sac-derived teratomas in C3H mice treated with busulphan*

| No. of animals | Treatment | No. of fectectomies | No. of tumors |
|---|---|---|---|
| 10 | 3 mg* | 32 | 15 |
| 10 | 5 mg | 29 | 13 |
| 10 | oil | 31 | 14 |

*Busulphan was injected i.p. (3 or 5 mg/100 g body weight) suspended in 0.2 ml arachis oil at day 3 of pregnancy.

The macroscopical and microscopical appearance of tumors was very similar to that observed in mice (Table 2).

4. *Development of teratomas in relation to the age of yolk sac*

In the 2 groups in which the mice were fetectomized either at day 11 or 13 and killed 3 weeks later, the multiple tumors which developed had the same macroscopic appearance. However, in the third group, fetectomized at day 15, only one cyst was recorded in a group of 10 mice.

The morphology of the tumors which developed from 11-day old yolk sac was similar to that described previously. In contrast, tumors developed from 13-day old yolk sac contained mostly endodermal cysts filled with mucin. This endodermal epithelium regularly formed folds similar to intestinal villi with a central core of connective tissue. Smooth muscle orientated in transverse and longitudinal layers was regularly seen around these endodermal cysts (Fig. 10). Often the formation of pancreas with Langerhans islets was observed around these intestinal-like structures.

Derivatives of other germ layers—ectodermal or mesodermal structures—although occasionally present, were less frequent. Skin with its derivatives, and cartilage plus bone, were seen in only one and two cases, respectively.

The only tumor recorded in mice fetectomized at day 15 of pregnancy, consisted of a cyst lined with endodermal epithelium.

In rats, tumors were found in all animals on which the operation had been performed, but in animals fetectomized on the 15th and 17th day the tumors had a more cystic appearance.

The teratomas which developed in both groups operated on at day 10 and 12 were morphologically identical; they contained all the previously described kinds of tissues [6, 7]. The occasional presence of small pieces of fetus near the yolk sac described previously after fetectomy performed at day 10 of pregnancy, did not modify the morphology of the tumors. Tumors obtained after fetectomy performed at the 15th day of pregnancy in rats were quite similar to those described in mice fetectomized at day 13: endodermal and intestinal-like cysts with occasionally seen squamous epithelium, cartilage and bone.

In the animals operated on at day 17, the results were similar to those obtained in rats fetectomized at the 15th day of pregnancy, but the cartilage and bone were found only in one case and no pancreatic tissue was observed (Table 2).

## DISCUSSION

The results of this experiment indicate that the displaced visceral yolk sac differentiates into teratomas not only in rats and hamsters [6, 12] but also in mice. From these findings it can be concluded that the capacity of the visceral yolk sac to differentiate into various adult tissues is a general phenomenon, common to most species of rodents. Moreover, we showed that the benign teratomas develop from the fetal membranes (extra-embryonal tissue) and not from germ cells or embryonal cells. Indeed, killing of primary germ cells by busulphan did not influence the appearance of teratomas or the incidence of various differentiated tissues in these tumors. This has previously been shown by us in rats [8] and has now been confirmed in the present experiment in mice.

Moreover, "contamination" with embryonal cells found when fetectomy was performed at day 10 of pregnancy, did not change the variety of tissues found in teratomas.

Since we have shown that these teratomas do not develop from germ cells [8] one must consider the totipotentiality of 11–12-day old visceral yolk sac. The capacity of this extra-embryonic membrane to give rise to fetal tissue will be not so surprising if one re-

members that the egg cylinder cleared of extra-embryonic parts and transplanted under the kidney capsule develops into teratomas containing parietal yolk sac and bone marrow [13]. As it is known that hemopoietic cells are derived from their precursors found in yolk sac [14], it is probable that this extra-embryonic membrane is formed in the teratomas by embryonal cells [4]. This suggests that determination of the embryonic and extra-embryonic parts is not irreversible.

This reversibility, however, can only be observed during embryo-genesis. Once the fetal development, mainly characterized by organo-genesis, has started (day 11 in mice and day 12 in rats), the visceral yolk sac loses its multipotentiality. From then on only endodermal and rare mesodermal derivatives are found in the visceral yolk sac-derived teratomas. Hence, it is clear that there exists a definite relationship between the age of the yolk sac and its differentiation potential. The more prolonged differentiation potential found for the rat visceral yolk sac compared to the mouse is reflected by the differences in morphology of this membrane at later stages. Indeed, whereas the mouse visceral yolk sac shows definite signs of degeneration at day 15, the cells of a 2-day older rat visceral yolk sac are still well preserved.

Compared to the induction of teratocarcinomas which is dependent upon the species (mouse) and the strain (C3H, 129 mice) used, the yolk sac-derived teratomas develop in all the different species (rat, hamster, mouse) and strains (BN and R rats, C57Bl and C3H mice) tested till now. The differences in differentiation potential of the visceral yolk sac amongst the two species (rat and mouse) examined here are linked to the morphological changes observed in the aging of this membrane.

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