INHIBITION OF GROWTH OF EMBRYONIC TISSUES TRANSPLANTED INTO SYNGENEIC NEWBORN RECIPIENTS

S. N. Zinzar, N. V. Karmanova, and G. Ya. Svet-Moldavskii

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Transplants of embryonic skin and teratomas formed after transplantation of minced tissues of embryos at the 18th-20th and 12-14th days of development into newborn syngeneic recipients grew much more slowly than similar grafts transplanted into adult recipients. It is postulated that factors controlling growth of embryonic tissues exist in embryos and newborn animals.

KEY WORDS: ectopic transplantation; embryonic tissues; age.

The growth of cysts formed from syngeneic grafts of the embryonic gastrointestinal tract (GIT) is depressed in newborn mice compared with that in adult recipients [1]. This depression is most marked in the case of cysts from the small intestine, but it is also found after transplantation of the embryonic stomach and large intestine into newborn animals. The question arises whether this phenomenon is specific purely for the embryonic GIT or whether it is a common feature of various embryonic tissues when grafted into newborn recipients.

Growth of embryonic skin and of a mixture of all embryonic tissue was studied after transplantation into newborn and adult mice.

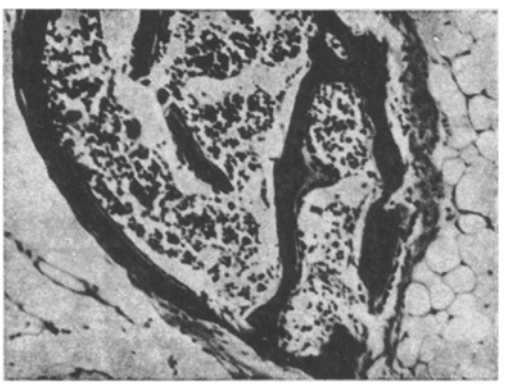

Fig. 1. Bone with bone marrow in graft from mixture of all embryonic tissues in adult recipient (4 months after transplantation). Here and in Figs. 2 and 3: hematoxylin–eosin, 112 ×.

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TABLE 1. Growth of Embryonic Skin Grafted into Newborn and Adult Mice (M ± m)

Recipient: age	sex	Time after transplantation: 1 month: strain	1 month: % in which cysts were found	1 month: mean weight (in g)	1 month: P	2½-3 months: strain	2½-3 months: % in which cysts were found	2½-3 months: mean weight (in g)	2½-3 months: P	4 months: strain	4 months: % in which cysts were found	4 months: mean weight (in g)	4 months: P
Newborn	♂	BALB/c	100	0,07± 0,02	0,01	DBA/2	76,9	0,03± 0,00	<0,01	F1(CBA×C57)i	9,09	0,02± 0,00	<0,01
Adult	♂	BALB/c	100	0,17± 0,04		DBA/2	100	0,44± 0,04		F1(CBA×C57)i	100	0,26± 0,05	
Newborn	♀	BALB/c	85	0,04± 0,01	0,01	DBA/2	70	0,03± 0,01	<0,01	F1(CBA×C57)i	20	0,004	
Adult	♀	BALB/c	100	0,31± 0,04		DBA/2	100	0,42± 0,04		F1(CBA×C57)i	—	—	

EXPERIMENTAL METHOD

Embryonic mice of both sexes of strains BALB/c and DBA/2 and (CBA × C57BL/6I)F_1 hybrids were used as the donors, and adult mice and young mice aged 1-3 days of both sexes and of the same strains were the recipients.

The skin of embryos aged 18-20 days was minced with scissors into pieces measuring 1-2 mm. Embryos aged 12-14 and 18-20 days also were cut up into similar pieces. Medium No. 199 was added to the minced tissue at the rate of 0.5 ml medium to 1.6 g tissue. The minced tissue was injected through a thick needle subcutaneously into newborn and adult recipients in a volume of 0.1 ml.

Regular observations were made on the time of appearance and growth of cysts. The mice were killed after 10 and 20 days and 1, 2, 3, and 4 months and the cysts were weighed and fixed for histological investigation. Sections were stained with hematoxylin–eosin

EXPERIMENTAL RESULTS AND DISCUSSION

1. Growth of Embryonic Skin Grafts. After transplantation of skin from embryos at the late stages of development into adult animals cysts consisting of a closed cavity filled with desquamated epithelium and hairs were formed. The cysts were found on the 5th-14th day after transplantation in 100% of the adult recipients and they increased in size steadily.

In the newborn animals the grafts could be seen under the skin on the day after transplantation and on the next 2 or 3 days, but later they could not be palpated in most of the animals.

At autopsy on the mice with skin grafts 10 days after injection, preserved pieces of transplanted material could be seen, arranged along the recipient's back. One or two cysts 1-2 mm in diameter were found 20 days and 1, 2, 3, and 4 months after injection in nearly all the animals. In some animals the cysts had completely disappeared by 3-4 months.

The comparative sizes (weights) of the cysts formed from embryonic skin in the newborn and adult animals 1, 2.5-3, and 4 months after transplantation and the percentage of cases in which they were found are given in Table 1. Clearly cysts were found at autopsy after 1 month in all the animals receiving grafts at the age of 1-2 days. At autopsy at the later periods cysts were found in a smaller percentage of cases, very much smaller after 4 months. The weight of the cysts in the newborn animals was several times less than the weight of cysts formed from the same quantity of transplanted material in the adult animals. The difference was statistically significant.

2. Growth of Grafts from Mixture of All Tissues of 12-14- and 18-20-Day Embryos. The results of transplantation of minced tissue of the whole embryo into newborn

TABLE 2. Growth of Grafts from Mixed Tissues of 12-14- and 18-20-Day Embryos in Newborn and Adult DBA/2 Mice (M ± m)

Donors	Recipient		Time after transplantation					
			2 1/2-3 months			4 months		
	age	sex	% in which cysts were found	mean weight (in g)	P	% in which cysts were found	mean weight (in g)	P
Embryos aged 18-20 days	Newborn	♂	93,8	0,07±0,01	<0,01	100	0,06±0,02	<0,01
	Adult	♂	100	1,22±0,16		100	0,34±0,06	
	Newborn	♀	89,5	0,04±0,01	<0,01	—	—	
	Adult	♀	100	0,91±0,14		—	—	
Embryos aged 12-14 days	Newborn	♂	100	0,08±0,02	<0,01	100	0,06	
	Adult	♂	100	0,54±0,13		100	2,42	
	Newborn	♀	100	0,02±0,01	<0,01	100	0,04	
	Adult	♀	100	0,62±0,13		100	1,04	

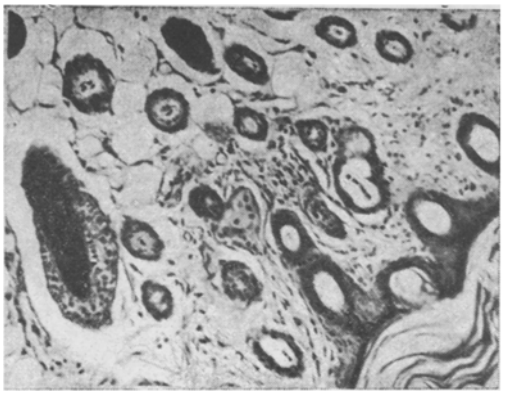

Fig. 2

Fig. 3

Fig. 2. Elements of skin in graft from mixture of all embryonic tissues in newborn recipient (4 months after transplantation).

Fig. 3. Graft from mixture of all embryonic tissues in adult recipient: cavity lined with cylindrical epithelium forming crypts and villi (4 months after transplantation).

and adult recipients were similar to those obtained by transplantation of embryonic skin. The results of these experiments are given in Table 2, which shows that the difference in weight of the cysts formed in the newborn and adult animals was greater than after transplantation of skin. The difference was increased in the case of transplantation of tissues from embryos aged 12-14 days (Table 2).

3. Histological Investigation of the Grafts. Histological investigation of the formation arising from the mixture of tissues of the whole embryo in newborn and adult mice showed that 4 months after transplantation they consisted of cartilage, bone with bone marrow (Fig. 1), connective tissue, and muscle. Stratified squamous epithelium was frequently found to line discrete cavities, as well as to form the epidermis of the skin, beneath which was a layer of dermis with hair follicles and sweat glands (Fig. 2). In a few cases cavities lined with cylindrical epithelium, with depressions sinking into the underlying connective tissue, beyond which was a layer of smooth muscles, could be seen. These were presumably cavities formed by intestinal tissue (Fig. 3). Gland cells with large amounts of secretion also were observed. Adipose tissue was abundant in all the grafts.

The formations obtained from grafts of embryonic tissues in the newborn animals were indistinguishable in morphology from those in the adults. The same tissues were found, but in the adults the mass of tissue was greater, and blood vessels and regions similar to intestinal tissue were more frequently seen.

The results of these experiments show that in newborn animals grafts consisting of embryonic tissues gradually diminished in size and most of the injected material was absorbed. Meanwhile, in adults the grafts gradually grew in size. In newborn animals growth of most embryonic tissues capable of growth in adult recipients was thus inhibited.

The mechanism of inhibition of growth of embryonic tissues in newborn recipients is not clear. The phenomenon is evidently not connected with an immunological response of the recipient to the graft. Transplantation was carried out under strictly syngeneic conditions into immunologically incompetent recipients. In the classical view, immunological incompetence of the recipient can only facilitate growth of embryonic grafts. Recently it has even been shown [2, 3] that grafts of embryonic tissues grow better in immunologically incompetent irradiated adult mice. Presumably in newborn animals either certain factors necessary for growth of transplants are absent, or in embryos and newborn animals there exist cellular and (or) hormonal factors which control the ability of embryonic cells to grow and differentiate. These factors or essential combinations of them are absent in adult animals.

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