Nonimmune and Immune Surveillance. I. Growth of Tumors and Normal Fetal Tissues Grafted Into Newborn Mice

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ABSTRACT—Growth of various fetal tissues and transplantable tumors in syngeneic newborn and adult mice [BALB/c, DBA/2, and (CBA × C57BL/6J)F1] was compared. Fetal skin, a mixture of all fetal tissues, and tumors were transplanted. The tumors arose spontaneously (hepatomas, mammary gland adenocarcinoma (MGAC)) or resulted from malignant conversion of ectopic transplants either of fetal tissues (urinary bladder carcinoma, adenocarcinoma of small intestine, stomach sarcoma) or of adult animal tissues (ovarian carcinoma) in the syngeneic system. The growth of fetal skin transplants and teratomas, which developed after transplantation of minced tissue from 18- to 20-day and 12- to 14-day fetuses, was considerably inferior in newborn syngeneic recipients, as compared with similar transplants in adults. Inhibition of tumor growth observed in newborn animals was manifested in prolongation of latent period before tumor node appearance and in slowing of growth rate of developed tumors. One of six tumors studied (MGAC) grew at the same rate in newborn and adult recipients. It was suggested that a special type of cellular and/or humoral mechanisms controlling tumor growth exists in newborns. The activity of such factors was conceived as based on fetal tumor antigens as targets. We assumed that weakly antigenic and strongly antigenic tumors behaved differently in respect to nonimmune and immune surveillance mechanisms.—J Natl Cancer Inst 57: 47–55, 1976.

Classic works (1–6) that started modern investigations of tumor immunity demonstrated obvious distinctions in antigenicity and immunogenicity of different tumors. Spontaneous tumors (4, 5, 7–9) and tumors induced with plastics (10, 11) and 2-acetaminofluorene (7) are negligibly antigenic; tumors induced with 3-methylcholanthrene, 7-benz[a]anthracene (4, 6–10), and particularly oncogenic viruses are considerably antigenic. Antigenic structures have certain functions in the cell. It may be assumed a priori that spontaneous and other low antigenic tumors behave differently than highly antigenic tumors, in respect to local cellular surrounding and various distant systems that provide the integrity of the body. But it has been consistently pointed out that the classic conception of immune surveillance formulated by Erlich (12) and developed by Thomas (13) and Burnet (14–16) is recently supported mainly in systems with highly antigenic tumors. To reinforce the immune surveillance theory such data as lymphoid infiltration in the tumor (17–20), enhancement of cancerogenesis, and tumor growth in connection with the reduction of immunologic reactivity (21) are referred to.

At the same time, there is abundant evidence for lacking, or insufficiency, or limited efficiency of immune surveillance (22–28). Ascertainment and experimental analysis of facts that render unlikely the theory of immune surveillance and do not fit into its framework are important to the correct assessment of mechanisms involved in tumor growth control. The evidence for tumor growth from inefficiency of immune surveillance is not as important as the evidence for those systems in which tumor growth is either unobserved or inhibited despite the lack of surveillance, e.g., in case of lack of immune competence, at least from the classic standpoint.

It is important to study, in particular, low antigenic spontaneous tumors with regard to what data on active surveillance are still scarce, since these tumors are experimental models closest to natural tumor diseases.

Detection of fetal antigens in tumors makes it relevant for researchers to draw certain parallels between the experiments on tumor and fetal tissue transplantation. In this laboratory (29), growth of syngeneic fetal gastrointestineal tract transplants in newborn animals was inferior to that in adults, a tendency especially obvious for small-intestine transplants. Moreover, we observed inhibition of growth of adenocarcinoma in the small intestines of newborn recipients (29). The other two tumors studied (leiomyosarcoma of small intestine and adenocarcinoma of large intestine) grew at the same rate in adult and newborn recipients.

Consequently, we studied in more detail the growth of various fetal tissue and tumor transplants in newborn recipients, as compared to adults. We used either spontaneous tumors or those which developed from malignant conversion of ectopic fetal or adult tissue transplants in the syngeneic system.

MATERIALS AND METHODS

Animals.—Male and female adult (8–12 wk old) and newborn (1, 2, 3, 5, 12, or 16 days old) mice of BALB/c, DBA/2, and (CBA × C57BL/6J)F1 strains were recipients. In the experiments on implantation of fetal tissues, donors were 12- to 14-day and 18- to 20-day fetuses of both sexes of the same mouse strains.

Fetal tissue implantation.—Twelve- to 14-day and 18- to 20-day fetuses and 18- to 20-day fetus skin were cut into 1- to 2-mm pieces, and 0.5 ml M199/1.6 g tissue was then added. In 0.1-ml volume, the minced tissue was injected sc through a thick needle into newborn and adult recipients.

Evaluation of results of implantation.—The time of appearance and the growth of cysts were noted. At 1, 2.5–3, or 4 months after implantation, mice were killed; cysts were dissected, freed from adherent tissue, and weighed. The significance of difference in the weights of the cysts was evaluated by Student's t-test. Cysts were

ABBREVIATIONS USED: M199 = medium 199; H & E = hematoxylin and eosin; HBSS = Hanks' balanced salt solution; MGAC = mammary gland adenocarcinoma.

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47

J NATL CANCER INST
fixed for histologic examination, sectioned, and stained routinely with H & E.

Tumors.—a) Stomach sarcoma (SS-1-72): The tumor arose in a BALB/c mouse as a result of spontaneous malignant conversion of syngeneic transplant tissue of a fetal stomach. It appeared 21.5 months after implantation; it was maintained in BALB/c males and females and accepted in 100% of adult mice. We used tumor from the fourth transplant generation. The tissue was minced in a metal homogenizer and diluted in HBSS at a concentration of 1 g/2 ml, with subsequent sc implantation in a 0.1-ml volume.

b) Urinary bladder carcinoma (UBC-1-73) (30): Poorly differentiated transitional cell carcinoma was obtained at spontaneous malignant conversion of a fetal urinary bladder syngeneic transplant in a (CBA × C57BL/6J)F1 mouse. It appeared 15 months after transplantation and grew progressively in syngeneic adult recipients; its growth was considerably inferior in the females, especially when the tumor was transplanted in pieces. We used tumor from the sixth, seventh, and eighth transplant generations. The tumor was transplanted into adult and newborn males either in 1-2×1-2-mm pieces or as suspension of tumor minced in a metal homogenizer and diluted in HBSS at a concentration of 1 g/ml.

c) Ovarian carcinoma (OC-1-72): Dr. T. K. Veskova obtained this granulocellular carcinoma from a female (CBA × C57BL/6J)F1 mouse, 10 months after an operation by Biskind's method (31). It was passaged three times in castrated females and subsequently maintained in noncastrated animals. We used tumor from the 3-7 and 3/10 transplant generations (the numerator and denominator denote the number of passages in castrated and noncastrated females, respectively). The tumor grew progressively in all syngeneic adult recipients. Tumor tissue was minced in a metal homogenizer and then diluted in HBSS at a concentration of 1 g/ml. In one experiment, the tumor was transplanted by single-cell suspension (1.2×107 cells/0.1 ml).

d) Hepatomas (H-1-73, H-2-73): These were supplied by Dr. T. V. Osipova. A spontaneous liver tumor was detected in a (CBA × C57BL/6J)F1 mouse killed 15 months after fetal syngeneic urinary bladder implantation. The tumor did not develop from the transplant, but it was maintained in adult hybrid mice. The first generation tumors grew slowly. H-1-73 and H-2-73 were transplanted 5 and 24 months after implantation, respectively. Later these tumors were maintained in adult hybrid females and males, with 100% acceptance. Ninth passage H-1-73 and fourth passage H-2-73 were used. The tissue was minced in a metal homogenizer, diluted in HBSS at a concentration of 1 g/ml (H-1-73) and 1 g/3 ml (H-2-73), and subsequently implanted sc in a 0.1-ml volume.

e) MGAC:MGAC was obtained from a spontaneous mammary gland tumor arising in a BALB/c female. This tumor normally grew progressively in all syngeneic adult recipients of both sexes. We used tumor from the 35th transplant generation. A single-cell suspension was prepared and injected sc in the volume of 0.1 ml (5×106 living cells unstained by eosin).

Evaluation of results of tumor transplantation.—Animals were examined twice weekly for tumor appearance. At completion of the experiments, the animals were killed; the tumors were removed, freed from adherent tissue, and weighed. The significance of difference in tumor weights was evaluated by Student's t-test.

Tumor freezing.—We froze the tumor cell suspension in M199 by adding 10% dimethyl sulfoxide until -40°C at a rate of 1°C/minute. The freezing rate between -40 and -80°C was 5°C/minute; over the range of -80 to -160°C, it was 20°C/minute (32). The tumor cell suspension was then stored in a liquid nitrogen freezer. Tumors were thawed in a 37°C water bath and transplanted; grown tumors were used in the experiments.

RESULTS

Growth of Fetal Tissue Implants

Growth of fetal skin implanted into newborn and adult mice

Cysts were detected in 100% of adult recipients 5-14 days after transplantation of fetal skin from the late term of pregnancy; they grew gradually. Close cavities were filled with desquamated epithelium and hair.

In newborn animals, implants were visible under the skin within 1-3 days after inoculation, but they were not palpable in most animals. At autopsy 10 days after inoculation, remnants of transplanted material were visible along the backs of recipients with skin grafts. Nearly all animals had 1 or 2 cysts 1-2 mm in diameter by 20 days and 1, 2.5, and 4 months after inoculation. Cysts disappeared completely in some animals within 3-4 months.

Table 1 summarizes the percent incidence and comparative size (weights) of cysts developed from fetal skin in newborn and adult animals at 1, 2.5, and 4 months after implantation. During dissection 1 month after transplantation, cysts were observed in nearly all adult mice (85.7% of females and 100% of males) that received implants as 1- to 2-day-old newborns and were observed in all adult recipients; at 2.5-3 and 4 months after transplantation, the percent incidence of cysts decreased. The weights of cysts in newborn animals were much less than those of cysts arising from the same amount of implanted material in adult animals. The difference in the weights of cysts formed from tissue implanted into newborn and adult animals was statistically significant.

Growth of mixture of all tissues from 12- to 14-day and 18- to 20-day fetuses implanted into newborn and adult mice

Results of implantation of minced tissue of a whole fetus into newborn and adult recipients were analogous to those obtained after fetal skin implantation. Results in table 2 show that the difference in the weights of cysts arising in newborn and adult animals was more significant than the difference after skin implantation. This weight difference was more pronounced after implantation of 12- to 14-day than after the 18- to 20-day fetal tissue.

Histologic examination of implants

Histologic examination of growths developed from a
TUMORS AND NORMAL FETAL TISSUES GRAFTED INTO NEWBORN MICE

Table 1.—Growth of fetal skin implanted into newborn and adult mice

<table>
<thead>
<tr>
<th>Recipients</th>
<th>Time after implantation</th>
<th>Incidence of cysts</th>
<th>Average wt, g</th>
<th>P⁸</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Age</td>
<td>Strain</td>
<td>mo</td>
<td>%</td>
</tr>
<tr>
<td></td>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Newborn</td>
<td>♂</td>
<td>BALB/c</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>Adult</td>
<td>♂</td>
<td>BALB/c</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>Newborn</td>
<td>♀</td>
<td>BALB/c</td>
<td>1</td>
<td>85.7</td>
</tr>
<tr>
<td>Adult</td>
<td>♀</td>
<td>BALB/c</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>Newborn</td>
<td>♂</td>
<td>DBA/2</td>
<td>2.5-3</td>
<td>76.9</td>
</tr>
<tr>
<td>Adult</td>
<td>♂</td>
<td>DBA/2</td>
<td>2.5-3</td>
<td>100</td>
</tr>
<tr>
<td>Newborn</td>
<td>♀</td>
<td>DBA/2</td>
<td>2.5-3</td>
<td>70</td>
</tr>
<tr>
<td>Adult</td>
<td>♀</td>
<td>DBA/2</td>
<td>2.5-3</td>
<td>100</td>
</tr>
<tr>
<td>Newborn</td>
<td>♂</td>
<td>(CBA × C57BL/6J)F</td>
<td>4</td>
<td>9.09</td>
</tr>
<tr>
<td>Adult</td>
<td>♂</td>
<td>(CBA × C57BL/6J)F</td>
<td>4</td>
<td>100</td>
</tr>
<tr>
<td>Newborn</td>
<td>♀</td>
<td>(CBA × C57BL/6J)F</td>
<td>4</td>
<td>20</td>
</tr>
<tr>
<td>Adult</td>
<td>♀</td>
<td>(CBA × C57BL/6J)F</td>
<td>4</td>
<td>—</td>
</tr>
</tbody>
</table>

*Mean±SE.
*By Student's t-test.

Table 2.—Growth of mixture of tissues of 12- to 14-day and 18- to 20-day fetuses implanted into DBA/2 newborn and adult mice

<table>
<thead>
<tr>
<th>Donors</th>
<th>Incidence of cysts, %</th>
<th>Average wt, g</th>
<th>P⁸</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.5-3</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>18- to 20-day fetuses</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Newborns</td>
<td>♂</td>
<td>93.8</td>
<td>0.07±0.01</td>
</tr>
<tr>
<td>Adults</td>
<td>♂</td>
<td>100</td>
<td>1.22±0.16</td>
</tr>
<tr>
<td>Newborns</td>
<td>♀</td>
<td>89.5</td>
<td>0.04±0.01</td>
</tr>
<tr>
<td>Adults</td>
<td>♀</td>
<td>100</td>
<td>0.91±0.14</td>
</tr>
<tr>
<td>12- to 14-day fetuses</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Newborns</td>
<td>♂</td>
<td>100</td>
<td>0.08±0.02</td>
</tr>
<tr>
<td>Adults</td>
<td>♂</td>
<td>100</td>
<td>0.54±0.13</td>
</tr>
<tr>
<td>Newborns</td>
<td>♀</td>
<td>100</td>
<td>0.02±0.01</td>
</tr>
<tr>
<td>Adults</td>
<td>♀</td>
<td>100</td>
<td>0.62±0.13</td>
</tr>
</tbody>
</table>

*Mean±SE.
*By Student's t-test.

mixture of all fetal tissues in newborn and adult mice 4 months after implantation revealed cartilage and a bone with bone marrow (fig. 1), and connective and muscle tissue. Stratified squamous epithelium lining some cavities (fig. 2) and epidermis over dermis with hair follicles and sudoriferous glands were frequent (fig. 3). In some cases, cavities were lined with columnar epithelium with hollows into connective tissue above a layer of smooth muscle. These cavities may be considered to be formed from intestinal tissue (fig. 4). Glandular cells with a large amount of secretion were also observed; all implants had much fat.

Growth of Tumors Implanted Into Newborn and Adult Mice

Stomach sarcoma

The mice were killed on day 14 after tumor transplantation. Tumor growth was significantly inhibited in mice receiving grafts at 1, 2, and 3 days of age (table 3).

Urinary bladder carcinoma

Inhibition of UBC-1-73 growth was most significant in newborns. The results of implantation of this tumor into animals of different ages are in table 3. A tumor transplant at sixth passage was grafted in pieces. The first tumors arose in adult males 23 days after implantation; 63 days after implantation, the incidence of tumors had reached 90.9%. When the mice were killed on day 84, the average tumor weight in adults was 6.5±0.9 g. The animals receiving a tumor transplant at the ages of 1 and 4 days did not develop tumors until the end of the observation period (84 days). Several mice receiving grafts at the ages of 12 and 16 days developed tumors. The time gap between the development of first tumors in males (receiving grafts at the age of 16 days) and in adult male recipients was 33 days.

The results obtained from the implantation of tumors from the seventh (grafting by pieces) and eighth (grafting by suspension) transplant generation into 1-day-old, 5-day-old, and adult males are in text-figure 1. In 1-day-old and 5-day-old newborns, tumors appeared 19 and 23 days later, respectively, than in adults. Tumor weight range was considerable: Tumor weight in adult males was 1.84±0.44 g, whereas in newborns it was 0.05±0.03...
### Table 3.—Growth of tumors in newborn and adult mice

<table>
<thead>
<tr>
<th>Tumors</th>
<th>Group No.</th>
<th>Strain</th>
<th>Age</th>
<th>Sex</th>
<th>Number</th>
<th>Incidence %</th>
<th>Average wt, g a</th>
<th>P b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stomach sarcoma (passage 4)</td>
<td>1</td>
<td>BALB/c</td>
<td>Adult</td>
<td>♂</td>
<td>12</td>
<td>100</td>
<td>1.48±0.24</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>BALB/c</td>
<td>1-day-old</td>
<td>♂</td>
<td>6</td>
<td>100</td>
<td>0.41±0.25</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>BALB/c</td>
<td>2-day-old</td>
<td>♂</td>
<td>17</td>
<td>100</td>
<td>0.52±0.11</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>BALB/c</td>
<td>3-day-old</td>
<td>♂</td>
<td>8</td>
<td>100</td>
<td>0.42±0.08</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>BALB/c</td>
<td>Adult</td>
<td>♀</td>
<td>10</td>
<td>100</td>
<td>1.95±0.18</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>BALB/c</td>
<td>1-day-old</td>
<td>♀</td>
<td>5</td>
<td>100</td>
<td>0.29±0.06</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>BALB/c</td>
<td>2-day-old</td>
<td>♀</td>
<td>8</td>
<td>100</td>
<td>0.58±0.13</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>BALB/c</td>
<td>3-day-old</td>
<td>♀</td>
<td>6</td>
<td>100</td>
<td>0.26±0.06</td>
<td></td>
</tr>
<tr>
<td>Urinary bladder adenocarcinoma (passage 6)</td>
<td>1</td>
<td>(CBA × C57BL/6J)F1</td>
<td>Adult</td>
<td>♂</td>
<td>11</td>
<td>90.9</td>
<td>6.5±0.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>(CBA × C57BL/6J)F1</td>
<td>1-day-old</td>
<td>♂</td>
<td>10</td>
<td>0.0</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>(CBA × C57BL/6J)F1</td>
<td>4-day-old</td>
<td>♂</td>
<td>5</td>
<td>0.0</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>(CBA × C57BL/6J)F1</td>
<td>12-day-old</td>
<td>♂</td>
<td>3</td>
<td>33.0</td>
<td>1.1</td>
<td></td>
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<tr>
<td></td>
<td>5</td>
<td>(CBA × C57BL/6J)F1</td>
<td>16-day-old</td>
<td>♂</td>
<td>3</td>
<td>66.0</td>
<td>2.3</td>
<td></td>
</tr>
<tr>
<td>Ovarian carcinoma (passage 3/10)</td>
<td>1</td>
<td>(CBA × C57BL/6J)F1</td>
<td>Adult</td>
<td>♀</td>
<td>22</td>
<td>100</td>
<td>5.52±0.22</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>(CBA × C57BL/6J)F1</td>
<td>1-day-old</td>
<td>♀</td>
<td>21</td>
<td>38</td>
<td>0.27±0.16</td>
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</tr>
<tr>
<td></td>
<td>3</td>
<td>(CBA × C57BL/6J)F1</td>
<td>Adult</td>
<td>♀</td>
<td>30</td>
<td>100</td>
<td>2.86±0.41</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>(CBA × C57BL/6J)F1</td>
<td>1-day-old</td>
<td>♀</td>
<td>24</td>
<td>33.3</td>
<td>0.12±0.06</td>
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<tr>
<td>H-2-73 (passage 4)</td>
<td>1</td>
<td>(CBA × C57BL/6J)F1</td>
<td>Adult</td>
<td>♀</td>
<td>14</td>
<td>100</td>
<td>3.3±0.09</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>(CBA × C57BL/6J)F1</td>
<td>1-day-old</td>
<td>♀</td>
<td>34</td>
<td>100</td>
<td>0.78±0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>(CBA × C57BL/6J)F1</td>
<td>Adult</td>
<td>♀</td>
<td>15</td>
<td>100</td>
<td>2.78±0.16</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>(CBA × C57BL/6J)F1</td>
<td>1-day-old</td>
<td>♀</td>
<td>29</td>
<td>100</td>
<td>0.77±0.02</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>(CBA × C57BL/6J)F1</td>
<td>Adult</td>
<td>♀</td>
<td>14</td>
<td>100</td>
<td>1.44±0.16</td>
<td>P&lt;0.01</td>
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<td></td>
<td>6</td>
<td>(CBA × C57BL/6J)F1</td>
<td>1-day-old</td>
<td>♀</td>
<td>31</td>
<td>100</td>
<td>0.50±0.02</td>
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<tr>
<td></td>
<td>7</td>
<td>(CBA × C57BL/6J)F1</td>
<td>Adult</td>
<td>♀</td>
<td>15</td>
<td>100</td>
<td>1.49±0.14</td>
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<tr>
<td></td>
<td>8</td>
<td>(CBA × C57BL/6J)F1</td>
<td>1-day-old</td>
<td>♀</td>
<td>20</td>
<td>100</td>
<td>0.51±0.04</td>
<td></td>
</tr>
</tbody>
</table>

* Mean±se.

a P<0.01 indicates difference between group No. 1 and group No. 3. Differences between other specific groups are similarly indicated. Calculated by Student's t-test.

g with 100% incidence of tumors in adults and newborns (both were killed 62 days after implantation of tumor from the seventh transplant generation).

### Ovarian carcinoma

The results of the experiments with OC-1-72 are in text-figure 2. In experiment 1, tumors developed in adults earlier than in newborns, and their growth was more progressive, especially in males. Adult males with large tumors (5–6 g) died 36 days after implantation. At this time, only 70% of tumor-bearing mice inoculated when newborns were alive. At their death 41 days after implantation, the average tumor weight for males inoculated while newborns was 1.24±0.33 g. Adult and newborn females were also killed 41 days after implantation. Tumor weights in adult females and females inoculated when newborns were 7.63±0.32 g and 1.51±0.33 g (P<0.01), respectively. Similar results were obtained in experiment 2 (text-fig. 2). Inhibition of tumor growth in mice inoculated when newborns was observed in experiment 3 (table 3), in which tumor from the 3/10 transplant generations was used: The tumor was transplanted by a single-cell suspension, and the animals were killed 28 days after tumor implantation.

### Hepatoma (H-1-73)

After the mice received grafts, tumors arose much later in newborns than in adults (text-fig. 3). When 100% of adults had developed large tumors, no tumors were found in mice inoculated while newborns.

### Hepatoma (H-2-73)

A significant inhibition of tumor growth was observed in mice inoculated while newborns (table 3).
planted into newborn recipients gradually diminished in size (tables 1, 2); in some newborns, cysts disappeared completely within 3–4 months (table 1), whereas their growth was rapid in adults. The phenomenon of growth inhibition was especially pronounced when slow-growing tumors were used, i.e., urinary bladder carcinoma, ovarian carcinoma, and one hepatoma (H-1-73). After implantation, these tumors arose in newborn recipients much later than in adults. The gap between the time of tumor appearance in newborns and adults varied from 15 to 30 days. In some cases no tumors developed in recipients inoculated at 1–4 days of age during the whole observation period (table 3). When tumor-bearing adults had very large tumors (5–6 g), the animals that received implants as newborns developed the first tumor nodes. For example, an increase in percent incidence of urinary bladder tumors and its direct dependence on the age when tumor implantation was performed can readily be observed (table 3). At transplantation of quickly progressing tumors, the time of tumor appearance in adults and newborns coincided. However, the rate of growth of such tumors in newborns was considerably lower than the tumor growth rate in adults. Weights of adenocarcinoma of the small intestine, stomach sarcoma, and H-2-73 were significantly less in newborns than in adults killed simultaneously.

Several factors may be responsible for the observed phenomenon, and immunologic factors may be the least important. Newborn mice are not yet immunologically competent. In particular, they respond to T-independent antigens (33) but they lack response to T-dependent antigens, which are tumor cell antigens (34). Many studies have shown that the lack of immune response in newborns makes it possible to graft even xenogeneic tumors into them (35-38). Due to the lack of immune competence, newborns respond to quickly progressing tumors, the time of tumor appearance coincided. How­ever, the rate of growth of such tumors in newborns was considerably lower than the tumor growth rate in adults. Weights of adenocarcinoma of the small intestine, stomach sarcoma, and H-2-73 were significantly less in newborns than in adults killed simultaneously.

Several factors may be responsible for the observed phenomenon, and immunologic factors may be the least important. Newborn mice are not yet immunologically competent. In particular, they respond to T-independent antigens (33) but they lack response to T-dependent antigens, which are tumor cell antigens (34). Many studies have shown that the lack of immune response in newborns makes it possible to graft even xenogeneic tumors into them (35-38). Due to the lack of immune response, newborns develop certain syngeneic tumors more easily (39). Again, there is evidence (40, 41) of accelerated appearance of syngeneic tumors in immunosuppressed mice. Therefore, one would expect stimulation of tumor growth in newborn mice in our experiments, but we obtained opposite results in five of six tumors studied. This is paradoxical in light of the immunosurveillance theory and gives us cause to suppose that some mechanisms other than the lack or insufficiency of immune reactions are mainly responsible for the observed phenomenon.

Surprisingly, tumors with the most pronounced inhibition of growth (urinary bladder carcinoma, H-1-73,
and ovarian carcinoma) arose only after recipients (newborn mice) became immunologically mature adults. In this case, it is conceivable, as Prehn (23) suggested, that some form of immune response to the tumor is necessary for its growth. Thus it may be assumed that in our experiments mice becoming immunologically competent began to produce antitumor antibodies that enhance tumor growth. Newborns do not develop tumors, since antibodies stimulating tumor growth are not produced. However, it was shown in a transplantable lymphoma (41), transplatable syngeneic melanoma (40), and a 3-methylcholanthrene-induced tumor (42) that the tumor induces a strong immune response in the host at the initial stages of tumor growth; the response decreases as the tumor reaches the least detectable sizes. Consequently, the greatest ability of the tumor to stimulate immune response coincides in our experiments with the least possibility of its realization (newborn period).

It is necessary to consider another assumption. Since during pregnancy the mother may come into contact with embryo-fetal antigens (43–45) and since multiparous females have immunity (cellular and humoral) to embryonic antigens, it may be assumed that humoral factors are transferred to embryos and newborns and that these factors are responsible for the observed phenomenon of inhibition of tumor growth in newborns. However, in these conditions, one should expect enhancement of tumor growth, as Kaliss and Dagg reported for pregnant mice (45). In experiments of tumor isografts and neutralization, no evidence has been obtained for a different response between virgin and multiparous mice. Neither lymphocytes nor serum from embryo-immune or multiparous mice caused any stimulatory or inhibitory effect on the admixed tumor cells (46, 47). Thus the phenomenon of tumor growth inhibition in newborn mice is difficult to analyze in light of the classic theory of immune surveillance (14–16) or from the standpoint of Prehn's hypothesis (23).

Local conditions, i.e., the rate of vascularization and proliferation activity of connective tissue in a tumor bed, are essential for the growth of transplantable tumor. There is no evidence that these processes are more intensive in adults than in newborns. On the contrary, vascular endothelium of adults is rather passive tissue renewing very slowly, with a labeling index of about 0.5% or less (48). Vascular endothelium of fetuses develop much faster, with a peak labeling index of 27% (49).

In our experiments, lack of inhibition of growth of some tumors in newborns—MGAC, leiomyosarcoma, and adenocarcinoma of large intestine (29)—with stimulation of tumor growth in the experiments of others (39) show that the activity of connective tissue of newborns is sufficient for the formation of stromal elements of the tumor. Inhibition of growth of normal fetal tissues, which usually contain vascular endothelium belonging to the donor tissue (48) and do not need connective tissue elements of the host for building up stroma (50), also testifies against the influence of lowered activity of connective and endothelial tissue on tumor growth.

Thus the aforementioned considerations eliminate suggestions about the role of immune factors, insufficient angiogenesis, or insufficient activity of connective tissue, which obviously do not account for inhibition of tumor growth in newborns; rather, such considerations make it relevant to assume the existence of other mechanisms regulating tumor growth. To us the observed phenomenon indicates the existence of nonimmune surveillance against neoplasia. We consider the idea that the effect of specific suppression of many cells by other cells is typical not only of T-suppressor lymphocytes described by Gershon et al. (51) but also is the manifestation of a general regulation inherent in cell systems, especially in embryogenesis.

No matter what the nature of factors in newborns and embryos that inhibit the growth of normal fetal tissues and tumors would be, one line of the regulation of growth of these tissues is probably realized through common fetal antigens. It is tumor tissue fetal antigens that are the targets for the embryonic factor controlling growth and differentiation. It is not apparent yet why tumor cells need fetal antigens. It has been suggested that fetal antigens on tumor cells are related to the function of the membrane that controls mitosis rather than specifically to neoplastic status. Fetal antigens were observed in adult mice on quickly multiplying testicular cells (52). It is conceivable that fetal antigens are those receptors on the cell membrane that control cell multiplication, which is necessary and consequently most marked in the fetal and early postnatal periods. Hence it would be relevant to isolate such factors during and at the end of the embryonic period. Possible detections of humoral factors controlling fetal antigen synthesis has been proved (53).

Thus there are two groups of syngeneic tumors: Growth of tumors belonging to one group is inhibited in newborn recipients, and growth of other tumors is equal in newborns and adults or accelerates later in newborns. The following tumors constitute the first group: spontaneous tumors (hepatomas) and the tumors which arise as a result of spontaneous malignant conversion of fetal and adult tissues [urinary bladder carcinoma, stomach sarcoma, ovarian granulocellular carcinoma (see experimental data presented above), adenocarcinoma of small intestine (21)]. Those constituting the second group are: MGAC, adenocarcinoma of large intestine, leiomyosarcoma (29), plasmacytoma (MOPC-460), which was chemically induced, and spontaneous adenocarcinoma (ADK-11) (39).

According to our preliminary data, simian virus 40-induced sarcoma grows at equal rates in newborn hamsters and adults.

A hypothesis which may find experimental proof is that these differences are correlated with absence or lack of strong antigens and particularly with the loss of normal H-2 antigens. Strong antigens accounted for by great changes, particularly in cell-surface structures, make the cell inaccessible to the effect of many factors of nonimmune surveillance, which are especially efficient in the embryonic period. To function like a tumor cell in this period, the cell should acquire new strong transplantation-type antigens. The same antigens are the
target for a particular mechanism defined as immune surveillance in the adult system. Low antigenic tumor cells may be regulated in the fetal and newborn periods and escape the effect of immune factors in the adult system; this is the reason for different growth of low and highly antigenic tumors in newborn and adult recipients. If Katz (54) and Fontalin et al. (55, 56) are right in assuming that H-2 locus antigens serve for integration in cellular interactions, then distortion or loss of H-2 antigen on the cell surface may function as "strong antigen."

A study of the complex of mechanisms of nonimmune surveillance is in progress.

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Figure 1.—Bone with bone marrow in graft from mixture of all fetal tissues in adult recipient, 4 months after implantation. H & E. x 112

Figure 2.—Cavity with stratified squamous keratinization of epithelium in graft from mixture of all fetal tissue in newborn recipient, 4 months after implantation. H & E. x 112
Figure 3.—Skin elements in graft from mixture of all fetal tissues in newborn recipient, 4 months after implantation. H & E. × 112

Figure 4.—Cavity lined with columnar epithelium forming crypts and villi. Mixture of all fetal tissues in adult recipient, 4 months after implantation. H & E. × 112