The Newborn Swiss Mouse as a Host for a Human Epidermoid Carcinoma (H.Ep. #3)

Transplantation and Chemotherapy Data*

PHILIP C. MERKER, MATTHEW BOWIE, AND PAULINE ANIDO

(Division of Tumor Biology, Sloan-Kettering Institute for Cancer Research, Sloan-Kettering Division Graduate School of Medical Sciences, Cornell University, New York, New York)

SUMMARY

A transplantable human epidermoid carcinoma (H.Ep. #3) has been successfully grown in newborn Swiss mice. When the tumor was inoculated into mice less than 24 hours old it was capable of growing for a limited period of time—approximately 14 days—before regressing. The growing tumor had no remarkable adverse effects on the bodily development of mice; however, a severe leukocytosis did develop in association with tumor growth. In addition, spleens and livers were significantly increased in weight, and there was marked activation of the macrophage population in the liver.

Data revealed that no uniform pattern of either a decrease or an increase in sensitivity to drug treatment could be predicted for newborn mice bearing human tumor transplants. In therapy trials with twenty chemicals, moderate antitumor effects were occasionally observed; however, they were always attended by severe toxicity to the newborn, and therefore these effects were judged to be not significant.

Transplantable human tumors growing in x-radiated and/or cortisone-treated mice (19), rats (33), and hamsters (8) have been used in experimental chemotherapy studies. However, chemotherapy results obtained from tumors grown in such conditioned hosts may be affected by the conditioning procedures used to allow the tumor to grow (25). Transplants of human tumor growing in the eyes of nonconditioned guinea pigs have been employed to study drug effects on human tumors (15), but this procedure has not been routinely used; and, although advantage has been taken of the immunological inadequacy of fetuses to grow human tissue in nonconditioned animals (14), no attempts have been made to evaluate anticancer agents in such systems.

Therefore, in view of our interest in the response of transplantable human neoplastic tissue to chemotherapy treatments, experiments were undertaken to study a human epidermoid carcinoma growing in neonatal mice. Potentially, this system offered the interesting advantage over a conditioned animal host that interpretations of test data need not be confounded by exogenous host-conditioning factors, an advantage already existent for heterologous tumor test systems which use the embryonated egg as host (10).

MATERIALS AND METHODS

The H.Ep. #3 tissue used in these studies is maintained in our laboratories as continuously passaged intramuscular tumors growing in x-radiated and cortisone-treated rats (36). Tissue suspensions for neonatal studies were prepared from 7- to 10-day-old tumors: surrounding muscle and necrotic debris were first removed; then the tumor was minced with scalpels and placed into a sterile glass homogenizer. Physiological saline (0.86 per cent), fortified with penicillin (1000 units/ml) and streptomycin sulfate (2 mg/ml), was then added in a volume equal to the weight of tissue. The

* Presented in part at the meeting of the American Association for Cancer Research, Chicago, Illinois, 1960.

This study was supported in part by research grant CY-5784 from the National Cancer Institute, National Institutes of Health, U.S. Public Health Service, Bethesda, Maryland; in part by contracts SA-43-ph-1925 and SA-49-ph-2443 from the Cancer Chemotherapy National Service Center, Silver Spring, Maryland.

Received for publication October 24, 1961.
moderately loose-fitting, ground-glass pestle was then gently forced into the tissue by hand. The tissue was hand-homogenized in this manner for 5–10 strokes, depending on the amount and quality of the tissue, until a relatively uniform dispersion was obtained. This procedure was accomplished at room temperature (24° C.) with no icing of tissue or solutions. The suspension was then poured from the homogenizer into a sterile petri dish while the pestle remained in place at the bottom of the mortar. The suspension which poured forth was of proper consistency and particle size to pass easily through a #23-gauge needle without the need for straining through gauze. One-ml. or 0.25-ml. tuberculin syringes, fitted with #23-gauge needles, were used to inoculate the tissue into newborn mice: the needle was first inserted intraperitoneally, then it was passed into the subcutaneous tissues where small volumes (0.01–0.10 ml.) were deposited at about the level of the shoulder.

Swiss mice were randomly bred in cages containing five females and one male. Several days prior to term, females were removed to individual cages after birth; mother and babies were housed in the same cage throughout the course of experimentation. Members of a litter were marked by cutting the tail. Since an average litter consisted of nine to ten babies, two to three experimental groups containing three to five newborns per group could be set up from one litter in a single cage. With a relatively small breeding colony of ten cages, ca. 30 litters per month were usable for experiments. Newborns given inoculations within 24 hours after birth were not handled again until they were 7 days old; thereafter, they were weighed, and the tumors were measured every other day. The mother was fed ad libitum a standard laboratory chow supplemented with sunflower seeds. Wood shavings were used as bedding.

Chemical agents used in therapy trials were generally prepared in 0.86 per cent sodium chloride; in the case of 6-mercaptopurine and 8-aza- guanine, 0.5 per cent carboxymethylcellulose made up in physiological saline was used as the suspending medium. Triethylenemelamine (TEM), bis(2-chloroethyl)methylamine (HN2); cytoxan; 1,6-dimethane sulfonyl-n-mannitol, and methotrexate were prepared fresh daily. Actinomycin D and mitomycin C were prepared as stock solutions, and when required for therapy, appropriate dilutions were made with saline. Final concentrations of all drugs were adjusted so that a dose was contained in a volume of approximately 0.1 ml. Therapy consisted in all cases of single, daily, intraperitoneal injections starting on the 7th day after the animals had been born and given inoculations of tumor. Treatment was then continued for 5 additional days.

RESULTS AND DISCUSSION

Growth and regression of tumors.—The amount of inoculum required for the successful growth of tumor depended on the transplantation line of H.Ep. #3 tumor. Two passage lines of H.Ep. #3 are maintained in our laboratories: a stock line, which had been in continuous intramuscular passage in x-radiated and cortisone-treated rats for approximately 3 years, and a repassaged line of tumor, which had been preserved in the frozen state for approximately 1 year prior to being repassaged in conditioned animals. This repassaged line has been maintained for the past 1½ years in our laboratories as an intramuscular tumor growing in conditioned rats.

Transplantation studies revealed that approximately a tenfold difference in growth potential exists between these two tumor lines: 0.10 ml. of 50 per cent suspension prepared from stock tumor produced within 12–14 days tumors with average diameters of 0.91 cm. (0.82–1.37 cm.), whereas a volume of 0.01 ml. of repassaged tumor was sufficient to produce approximately the same size tumors, if not slightly larger—1.12 cm. (0.40–1.80 cm.) within an equivalent period. A difference in growth potential between these two transplant lines has also been observed when the cortisone-treated young mouse (15–18 gm.) was used as a host for tumor inocula.

The difference in vitality of the two H.Ep. #3 tumor transplant lines need not necessarily be ascribed to the preservation of one of the lines as a frozen tissue. The divergence of a transplanted tumor into two or more lines, each of which has a different growth potential, has been recorded by Schrek (28) and Strong (32), and discussed by Klein (13). In addition, Toolan et al. (38) have demonstrated that preservation need not alter the characteristics of human tumors, and Hauschka (11) has established that frozen tumors can retain their original growth characteristics.

Regardless of the tumor passage line used, mice less than 24 hours old allowed the highest percentage of inoculated tumor to “take” (Table 1).

Tumors grown in newborns less than 24 hours old attained peak size within 14 days; thereafter, they remained static and/or regressed (Table 2). Occasionally a few tumors persisted and grew up to 20 days, but these were rare instances. At peak

1 Millerton Farms, Millerton, New York.
growth, tumors were round masses, firmly attached to the skin and chest wall, and consisted of well vascularized tissue with little or no central necrosis. The tumors did not usually ulcerate through the skin, nor did they grow by extension into the thoracic or abdominal cavities.

Histological examination of 14- to 15-day-old tumor revealed viable tissue with very little necrosis. There was negligible host inflammatory infiltration at this time. Mitotic frequencies were about 2–5 figures per high-power field. After a 2-week period tumors became increasingly necrotic, with greater amounts of host inflammatory reaction tissue. By the 20th day there was usually a complete absence of tumor tissue. Tumor recovered from 10- to 14-day-old mice grew exceedingly well on back-transplantation into conditioned young female rats or mice.

In some instances tumors were observed on the 7th to 8th day after transplantation in mice that were given inoculations when 2 to 5 days old; however, these tumors invariably remained static or had become smaller in size by the 14th–15th days (Chart 1). Histological examination revealed host inflammatory reaction tissue, necrosis, and few tumor cells. When 6- to 9-day-old mice were given

**TABLE 1**

**RELATIONSHIP BETWEEN AGE OF NEWBORN AND GROWTH OF H.Ep.#3**

<table>
<thead>
<tr>
<th>Age at implant</th>
<th>No. dead or missing/total no. injected</th>
<th>No. tumors/no. mice implanted</th>
<th>Av. tumor diameter (cm.)</th>
<th>Range of diameter</th>
<th>No. regressing tumors/total no. tumors</th>
<th>No. static tumors/total no. tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;24 hr.</td>
<td>12/34</td>
<td>19/22</td>
<td>1.12</td>
<td>0.65–1.55</td>
<td>4/19</td>
<td>4/19</td>
</tr>
<tr>
<td>2–5 days</td>
<td>9/24</td>
<td>4/15</td>
<td>0.78</td>
<td>0.70–1.00</td>
<td>4/4</td>
<td>0/4</td>
</tr>
<tr>
<td>6–9 days</td>
<td>18/40</td>
<td>2/22</td>
<td>0.65</td>
<td>0.60–0.70</td>
<td>2/2</td>
<td>0/2</td>
</tr>
<tr>
<td>14–17 days</td>
<td>10/25</td>
<td>3/15</td>
<td>0.65</td>
<td>0.60–0.70</td>
<td>2/2</td>
<td>1/3</td>
</tr>
</tbody>
</table>

Based on observations made within a 14- to 15-day period after tumor inoculation. Total number of litters used was six.

* Those tumors which had decreased in size or totally disappeared as judged by measurements made from the 7th to 14th–15th days.

† Those tumors which had not increased in size as judged by measurements made from the 7th to 14th–15th days.

**TABLE 2**

**GROWTH AND REgression OF H.Ep.#3 IN NEONatal SWISS MICE**

<table>
<thead>
<tr>
<th>Observation</th>
<th>9–10</th>
<th>12</th>
<th>14–15</th>
<th>17–19</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dead or missing (cumulative):</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Growing tumors:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. tumors observed/no. survivors</td>
<td>19/26</td>
<td>12/25</td>
<td></td>
<td>3/19</td>
<td>1/12</td>
</tr>
<tr>
<td>Average diameter (cm.)</td>
<td>0.88</td>
<td>1.08</td>
<td>1.10</td>
<td>1.15</td>
<td>1.15</td>
</tr>
<tr>
<td>Range</td>
<td>(0.70–1.15)</td>
<td>(0.70–1.15)</td>
<td>(1.00–1.30)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Static tumors:†</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. tumors observed/no. survivors</td>
<td>2/25</td>
<td></td>
<td>6/23</td>
<td>7/19</td>
<td>4/12</td>
</tr>
<tr>
<td>Average diameter (cm.)</td>
<td>0.85</td>
<td>1.03</td>
<td>0.96</td>
<td>0.86</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>(0.80–0.90)</td>
<td>(0.95–1.20)</td>
<td>(0.65–1.35)</td>
<td>(0.55–1.35)</td>
<td></td>
</tr>
<tr>
<td>Regressing or regressed tumors:‡</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. tumors observed/no. survivors</td>
<td>1/25</td>
<td></td>
<td>3/23</td>
<td>6/19</td>
<td>6/12</td>
</tr>
<tr>
<td>Average diameter (cm.)</td>
<td>0.60</td>
<td>0.70</td>
<td>0.98</td>
<td>0.88</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>(0.65–0.80)</td>
<td>(0.80–0.90)</td>
<td>(0.80–0.90)</td>
<td>(0.80–0.90)</td>
<td></td>
</tr>
<tr>
<td>No tumor growth</td>
<td>7/26</td>
<td></td>
<td>3/23</td>
<td>3/23</td>
<td>2/12</td>
</tr>
<tr>
<td>Per cent nontake</td>
<td>12%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Tumor implanted when mice were 24 hours old.
† Tumors which had not increased in size from the previous measurement.
‡ Tumors which had decreased in size from the previous measurement.
Data pooled from six litters.
transplants of H.Ep. #3, tumors were never observed to increase in size beyond a 0.6-cm. to 0.7-cm. bleb; histological examination always revealed necrotic tissue.

The requirement that a newborn less than 24 hours old be used as a recipient to obtain the highest percentage of successful tumor takes is in accord with the findings of Bullock (2), Gheorghiu (6), and Patti and Moore (26), who used mouse tumors transplanted to neonatal rats. The sequence of tumor growth which takes place within a 2-week period, followed by regression which we have observed with human tumor tissue, is essentially the same as for animal-tumor heterotransplants made into newborns (2, 6, 26).

These observations are consistent with the immunological characteristics of newborn mice (1, 29).

Although the newborn mouse is immunologically incompetent, it does not necessarily follow that all inoculated tumors will grow indiscriminately. In the present series of experiments inocula of H.Ep. #3 have been grown successfully in newborn Swiss mice. However, Kutner and Southam (16) have reported that another human epidermoid carcinoma (H.Ep. #2) was not amenable to transplantation into newborn mice. The absence of satisfactory tumor growth in one strain of mouse does not preclude the successful use of other strains of mice. Recently, Rothfels and co-workers (27) have demonstrated the practicability of exploring a variety of hybrid F1 newborn mice for transplantation studies.

Effect of tumor transplantation on newborn mice. —It was mentioned above that a difference was noted between the two transplant lines in the amount of tumor inoculum required to produce tumors of a comparable size. Similarly, a difference exists between the two H.Ep. #3 lines in respect to "killing" power: 0.1 ml. of 50 per cent stock tumor suspension, when injected subcutaneously into newborns less than 24 hours old, caused approximately 22 per cent of inoculated mice to die within the first 11- to 13-day period, whereas this same volume of inoculum prepared from the repassed tumor caused approximately 50 per cent of inoculated mice to die. By the 18th to 21st day, cumulative mortality was 40 per cent for stock tumor-inoculated mice and 80 per cent for repassed tumor-inoculated mice, respectively. A decrease in inoculum size of the repassed tumor to 0.05 ml. resulted in the death of 16 per cent of the mice within the first 11- to 13-day period, with cumulative mortality of 46 per cent by the 18th-21st day. This represents a twofold difference in "potency" between repassage and stock H.Ep. #3 tumor lines. A further decrease in inoculum size of repassaged tumor to 0.01 ml. produced 7 per cent mortality for the first 11-13 days, with a cumulative mortality value of 25 per cent by the 18th-21st days.

The presence of growing tumors did not necessarily affect body weight gain. In a typical littermate experiment, presented in Chart 2, it can be seen that the gains in body weight of mice given inoculations of tumor when less than 24 hours old were not significantly different from their tumor-free littermates. Pooled data collected on approximately 300 newborn mice given inoculations of tumor when less than 24 hours old revealed that the average body weight of 7-day-old tumor-bearers ranged from 3.8 gm to 6.1 gm. This was within the range of 4.0-6.8 gm. average body weight found for tumor-free 7-day-old mice. Average body weights for tumor-bearers on the 12th-14th days ranged from 5.5 to 10.8 gm., values not significantly different from a range of 6.1-11.8 gm. obtained for tumor-free mice of comparable age.

In contrast to the drastic effects which injections of homologous spleen can have on newborn
animals (31), and the mongolism which can be induced by extracts of human tumors in newborn hamsters (37), growth and regression of H.Ep. #3 transplants had no remarkable, grossly visible, adverse effects on surviving mice. However, data presented in Table 3 indicate that, by the 7th to 8th days, tumor-bearing suckling mice had developed a severe leukocytosis, and spleen weights were threefold and liver weights were approximately twofold heavier than organs of tumor-free suckling mice; by the 17th–18th days spleen weights were approximately equal for tumor-bearing and tumor-free mice; and the difference in liver weights between the two groups was less marked.

The leukocytosis observed in newborn mice is in essence similar to the reaction observed in adult mice bearing growing transplanted, spontaneous, or induced tumors (39). The remarkable enlargement of the spleen and liver observed in human tumor-inoculated newborns is reminiscent of the stimulation of embryonic liver growth by homologous tissue implants observed by Weiss (40), and the increase in liver and spleen weights reported for adult animals bearing transplanted (22, 39) and spontaneous tumors (4, 39). An increase in organ weights of tumor-bearing animals has been ascribed by Simonsen and co-workers (30) to an incompatibility of tumor and host.

Observations made in collaboration with Dr. L. J. Old on liver tissue, with the aid of histochemical procedures (7), revealed that the number of acid phosphatase-containing macrophages, as well as the amount of acid phosphatase per cell in the livers of tumor-bearing sucklings, was markedly increased as early as 2 days after tumor inoculation, and the increase persisted for at least 18 days (Figs. 1 and 2).

Cell-free extracts prepared from viable H.Ep. #3 tumor tissue also produced an increase in the toxic dose levels; a minimum of two experiments was performed at each dose level. A maximum tolerated dose in the newborn mouse chemotherapy studies was defined as the highest dose which, when repeated daily as a single intraperitoneal injection for 6 days, allowed mice to live for at least 6 days after the last dose of drug had been given. Although definitions for the maximum tolerated dose may vary for different test systems, an attempt was made to compare maximum tolerated doses obtained in newborn Swiss mice bearing growing human tumors with those obtained from adult Swiss mice bearing Sarcoma 180 implants and cortisone-conditioned mice bearing H.Ep. #3 tumors (Table 4).

Data reveal that no uniform pattern of either a decrease or increase in sensitivity to drug treatment can be predicted for newborn mice bearing human tumor transplants. For two of the drugs, N-desacetyltiocolchicine and Miracil D, data illustrate that newborns tolerate these chemicals

### Table 3

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Mice</th>
<th>Av. body wt. (gm.)</th>
<th>Av. WBC</th>
<th>Av. spleen wt. (mg.)</th>
<th>Av. liver wt. (mg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Tumor-bearers</td>
<td>2.1 (1.9–2.3)</td>
<td>2,406</td>
<td>8.9 (6.9–12.9)</td>
<td>110.6 (89.4–137.4)</td>
</tr>
<tr>
<td></td>
<td>Tumor-free</td>
<td>2.1 (1.9–2.3)</td>
<td>4,188</td>
<td>6.0 (5.7–6.7)</td>
<td>89.5 (83.8–100.8)</td>
</tr>
<tr>
<td>7–8</td>
<td>Tumor-bearers</td>
<td>5.4 (4.9–6.2)</td>
<td>54,000</td>
<td>70.0 (49.5–92.1)</td>
<td>284.5 (232.7–387.8)</td>
</tr>
<tr>
<td></td>
<td>Tumor-free</td>
<td>4.9 (4.0–5.5)</td>
<td>3,350</td>
<td>39.1 (27.3–51.2)</td>
<td>170.8 (131.4–210.3)</td>
</tr>
<tr>
<td>12–13</td>
<td>Tumor-bearers</td>
<td>7.5 (6.1–9.7)</td>
<td>89,800</td>
<td>92.5 (71.7–135.7)</td>
<td>410.2 (381.6–529.7)</td>
</tr>
<tr>
<td></td>
<td>Tumor-free</td>
<td>6.8 (5.5–7.9)</td>
<td>2,736</td>
<td>52.8 (23.5–43.7)</td>
<td>230.8 (171.1–263.9)</td>
</tr>
<tr>
<td>17–18</td>
<td>Tumor-bearers</td>
<td>10.9 (9.2–12.9)</td>
<td>22,567</td>
<td>144.5 (104.8–169.0)</td>
<td>688.8 (580.0–770.0)</td>
</tr>
<tr>
<td></td>
<td>Tumor-free</td>
<td>13.6 (11.5–16.2)</td>
<td>3,214</td>
<td>196.5 (85.3–211.1)</td>
<td>742.8 (615.3–970.0)</td>
</tr>
</tbody>
</table>
to a lesser extent than do either adult normal mice bearing a homologous tumor or cortisone-treated mice bearing a human tumor implant. Data for 5-FU illustrate that H.Ep. #3 tumor-bearing newborns and adult Swiss mice growing S-180 tumor tolerate essentially the same dose level, but that cortisone-treated, human tumor-bearing mice are less tolerant of the chemical. On the other hand, actinobolin sulfate and actidione are two drugs which are tolerated by normal Swiss mice and cortisone-conditioned mice bearing tumors at essentially the same dose level, whereas newborn mice are less tolerant of the chemicals. Although the magnitude of the difference in tolerance to actinobolin is approximately of the same order (4-5 X) as the difference in body weight of 7- to 12-day-old sucklings and young adult mice, it is still intriguing to ascribe this difference in tolerance to less favorably inclined detoxification (21) and/or excretion (17) mechanisms of the newborn.

Data for a single experiment at what was established to be the maximum tolerated dose are presented in Table 5 for twenty chemical agents. For the majority of drugs a doubling of the dose listed in the table resulted in death within 6 days after the start of treatment.

Moderate antitumor effects were occasionally observed, as in the case of 6-MP at 200 mg/kg. Data show that tumor growth was inhibited; however, at this dose all treated mice were dead 1 week after the end of therapy. It is doubtful whether antitumor effects observed under these circumstances have any significance. An attempt was made to alter the course of 6-mercaptopurine toxicity by treating suckling mice with hypoxanthine. Injections of hypoxanthine at 200 mg/kg did not prevent deaths or modify the severity of diarrhea produced in sucklings by 6-mercaptopurine. Amethopterin was also observed to slightly inhibit tumor growth and cause tumor regressions—however, always at doses which caused lethals.

The general inability of therapy to affect H.Ep. #3 growing in neonatal mice is in contrast to the anti-H.Ep. #3 activities displayed by several of these compounds when the tumor was grown in other animal hosts. Most notable is the remarkable anti-H.Ep. #3 effects of nitrogen mustard observed by Dagg and co-workers (3) and TEM by Harris (9) when explants of H.Ep. #3 were grown in the embryonated chicken egg. However, these same agents were classified as "inactive" when tumor was grown and tests conducted in the cortisone-treated mouse (19) and rat (34). A second notable exception between the results obtained from the newborn system and other animal hosts bearing human tumors is the case of actinobolin.

In tests conducted in the suckling mouse, the antibiotic was without effect; however, test data obtained when this tumor was grown in the conditioned rat (33) demonstrated that the antibiotic displayed good antitumor activity. Chemotherapy data on actinobolin obtained from the H.Ep. #3-conditioned Swiss mouse system (20) tend to confirm those of the suckling mouse rather than the rat host.

A third compound for which test data differ is 6-mercaptopurine: whereas test data obtained from rat (34) and mouse (19) H.Ep. #3 test systems tend to indicate that this antimetabolite inhibits tumor growth, results presented for the suckling mouse reveal that the compound is without antitumor activity. In single trials, 6-MP as well as HN2 appeared to allow tumors to grow and persist for a longer period than is usual. The data to support this observation are not conclusive; however, if the observation is correct it would be similar to that of Meeker and co-workers (18), who reported on the capacity of 6-MP to prolong the survival of skin homografts in rabbits. The mechanism, in the case of the newborn mouse-human tumor system, may not necessarily be the same.

A variety of factors operating singly or in combination may be responsible for the lack of positive correlation between the test data obtained from suckling mice and other host animals. In terms of host metabolic background, it is well established that newborn animals do not have the same general bodily metabolic rate as older animals (19), and that renal excretion (17) and drug detoxification mechanisms (5, 21) may be markedly different in the young. Host conditioning, which is necessary to allow heterotransplants to grow in young mice and rats, has been shown by Palm and co-workers (25) to affect tumor response to drug

### Table 4

<table>
<thead>
<tr>
<th>Compound</th>
<th>Newborn mouse, H.Ep. #3 (mg/kg)</th>
<th>Swiss mouse, Sarcoma 180 (mg/kg)</th>
<th>Conditioned Swiss mouse, H.Ep. #3 (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-Fluorouridine (5-FU)</td>
<td>60.0</td>
<td>50.0</td>
<td>18.0</td>
</tr>
<tr>
<td>Mircal D</td>
<td>25.0</td>
<td>125.0</td>
<td>62.5</td>
</tr>
<tr>
<td>N-desacetylthiocolchicine</td>
<td>2.0</td>
<td>80.0</td>
<td>10.0</td>
</tr>
<tr>
<td>Actinobolin sulfate</td>
<td>250.0</td>
<td>1000</td>
<td>1500</td>
</tr>
<tr>
<td>Actidione</td>
<td>2.5</td>
<td>40.0</td>
<td>30.0</td>
</tr>
</tbody>
</table>

* Millerton Farms; initial weight, 15-18 gm.
† Millerton Farms; conditioned on day of transplant with 150 mg/kg cortisone, single subcutaneous dose; initial weight, 15-18 gm.
### TABLE 5

**EFFECT OF DRUG TREATMENT ON H.Ep. #3 TUMOR, GROWING IN NONCONDITIONED, NEONATAL SWISS MICE**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose (mg/kg)*</th>
<th>Mortality</th>
<th>Av. Body weight (g.m.)</th>
<th>Av. Tumor diam. (cm.)</th>
<th>No. Regressing Tumors</th>
<th>No. Static Tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>T</td>
<td>C</td>
<td>T</td>
<td>C</td>
<td>T</td>
</tr>
<tr>
<td>TEM</td>
<td>1.0†</td>
<td>12</td>
<td>0/4</td>
<td>0/4</td>
<td>5.1</td>
<td>6.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>16</td>
<td>4/4</td>
<td>1/4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urethan</td>
<td>500†</td>
<td>12</td>
<td>0/3</td>
<td>0/3</td>
<td>6.2</td>
<td>6.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>19</td>
<td>2/3</td>
<td>0/4</td>
<td>7.5</td>
<td>9.7</td>
</tr>
<tr>
<td>1-Phenylene diamine HCl</td>
<td>100†</td>
<td>18</td>
<td>1/4</td>
<td>0/5</td>
<td>6.6</td>
<td>8.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>17</td>
<td>3/4</td>
<td>1/5</td>
<td>8.0</td>
<td>10.1</td>
</tr>
<tr>
<td>8-Azaguanine</td>
<td>75</td>
<td>12</td>
<td>0/4</td>
<td>0/4</td>
<td>6.2</td>
<td>6.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>19</td>
<td>1/4</td>
<td>0/4</td>
<td>8.6</td>
<td>8.8</td>
</tr>
<tr>
<td>Desacetylthiocholchicine</td>
<td>2.0</td>
<td>13</td>
<td>0/3</td>
<td>0/3</td>
<td>6.7</td>
<td>7.4</td>
</tr>
<tr>
<td>1,6-Dimethane sulfonyl-D-mannitol</td>
<td>500</td>
<td>12</td>
<td>0/3</td>
<td>0/4</td>
<td>7.1</td>
<td>8.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>19</td>
<td>2/3</td>
<td>1/4</td>
<td>7.5</td>
<td>10.7</td>
</tr>
<tr>
<td>Cytoxan</td>
<td>37.5</td>
<td>14</td>
<td>0/3</td>
<td>0/3</td>
<td>5.8</td>
<td>6.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>18</td>
<td>0/4</td>
<td>0/4</td>
<td>6.3</td>
<td>7.9</td>
</tr>
<tr>
<td>HN2</td>
<td>1.5</td>
<td>12</td>
<td>0/5</td>
<td>0/5</td>
<td>4.7</td>
<td>6.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>16</td>
<td>2/5</td>
<td>0/5</td>
<td>5.8</td>
<td>8.5</td>
</tr>
<tr>
<td>Actidione</td>
<td>2.5</td>
<td>14</td>
<td>0/3</td>
<td>0/4</td>
<td>7.2</td>
<td>7.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>18</td>
<td>0/3</td>
<td>1/4</td>
<td>8.7</td>
<td>9.7</td>
</tr>
<tr>
<td>6-Mercaptopurine</td>
<td>900</td>
<td>12</td>
<td>0/4</td>
<td>0/4</td>
<td>6.8</td>
<td>7.0</td>
</tr>
<tr>
<td>5-Fluorouracil</td>
<td>60</td>
<td>12</td>
<td>0/4</td>
<td>0/5</td>
<td>5.4</td>
<td>6.9</td>
</tr>
<tr>
<td>2,2'-Ethylene bis(4-amino-6-hydroxypyrimidine)</td>
<td>125</td>
<td>13</td>
<td>0/4</td>
<td>1/4</td>
<td>8.7</td>
<td>9.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>17</td>
<td>1/4</td>
<td>1/4</td>
<td>9/4</td>
<td>9.9</td>
</tr>
<tr>
<td>Mitomycin C</td>
<td>2.0</td>
<td>12</td>
<td>0/4</td>
<td>0/5</td>
<td>6.5</td>
<td>6.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>19</td>
<td>2/4</td>
<td>1/5</td>
<td>7.7</td>
<td>6.8</td>
</tr>
<tr>
<td>Actinomycin D</td>
<td>0.1</td>
<td>12</td>
<td>0/4</td>
<td>0/4</td>
<td>6.2</td>
<td>6.4</td>
</tr>
<tr>
<td>Actinobolin sulfate</td>
<td>250</td>
<td>13</td>
<td>0/3</td>
<td>0/3</td>
<td>5.5</td>
<td>6.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>18</td>
<td>1/3</td>
<td>1/3</td>
<td>6.8</td>
<td>8.4</td>
</tr>
<tr>
<td>Miracil D</td>
<td>250†</td>
<td>12</td>
<td>0/4</td>
<td>1/4</td>
<td>4.8</td>
<td>6.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>19</td>
<td>4/4</td>
<td>1/4</td>
<td>6.1</td>
<td>9.1</td>
</tr>
<tr>
<td>Azaserine</td>
<td>150†</td>
<td>12</td>
<td>1/4</td>
<td>0/3</td>
<td>6.1</td>
<td>9.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>19</td>
<td>4/4</td>
<td>0/3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-Fluoro-2'-deoxyuridine</td>
<td>160†</td>
<td>13</td>
<td>2/4</td>
<td>0/5</td>
<td>6.0</td>
<td>6.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>4/4</td>
<td>1/5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-Amino-6-(1'-methyl-4'-nitro-5'-imidazoyl)-thiopurine</td>
<td>7.5</td>
<td>13</td>
<td>0/4</td>
<td>0/4</td>
<td>8.6</td>
<td>9.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>2/4</td>
<td>0/4</td>
<td>10.2</td>
<td>10.8</td>
</tr>
<tr>
<td>Methotrexate (Amethopterin)</td>
<td>2.0</td>
<td>12</td>
<td>0/4</td>
<td>0/4</td>
<td>5.7</td>
<td>6.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>16</td>
<td>4/4</td>
<td>0/4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Started on 7th day and represents one-half the dose which caused all newborns to die within 5 days.
† Post-transplantation and age of mice.
‡ Delayed lethality (16-19 days).
treatment; therefore, test data arising from a non-
conditioned host, the suckling mouse, may differ
from those obtained from conditioned hosts be-
cause of the absence of conditioning. In suckling
mice, H.Ep. #8 tissue was transplanted subcuta-
neously, whereas in the young, conditioned mouse
tumor is inoculated intramuscularly, and in the
rat host the tumor is usually grown subcutaneous-
ly. In addition, the interval of time between tumor
implantation and the start of treatment may play
a role in tumor response to therapy. In the suck-
ling mouse, tests were started 7 days after tumor
inoculation when tumors are well established,
whereas, in the conditioned rat and mouse, ther-
apy is usually started 24 hours after tumor inocu-
lation. It is known, in general, that, when therapy
is started after transplanted tumors are well estab-
lished, treatment is normally less effective.

ACKNOWLEDGMENTS

The authors wish to thank Dr. George W. Woolley for his
kind support.

The authors wish to thank the following companies for
generous supplies of chemicals: Miraci! D, 6-mercaptopurine,
and 5-fluorouracil and 5-fluoro-2'-deoxyuridine were supplied by
Merck, Sharp and Dohme; actidione by the John Co.; and
azaserine and actinobolin sulfate by Parke-Davis and Co.; and
HN2, urethan, actinomycin D were supplied by
Burroughs Wellcome; TEM, methotrexate, 8-aza-
ty. In addition, the interval of time between tumor
implantation and the start of treatment may play
a role in tumor response to therapy. In the suck-
ling mouse, tests were started 7 days after tumor
inoculation when tumors are well established,
whereas, in the conditioned rat and mouse, ther-
apy is usually started 24 hours after tumor inocu-
lation. It is known, in general, that, when therapy
is started after transplanted tumors are well estab-
lished, treatment is normally less effective.

REFERENCES

1. BILLINGHAM, R. E.; and BRENT, L. A Simple Method for
Inducing Tolerance of Skin Homografts in Mice. Trans-

2. BULLOCK, W. F. Heterologous Transplantation: Mouse
Tumors in Rats. Lancet, 1:701–8, 1915.

3. DARG, C. P.; KANWIS, D. A.; TOOLAN, H. W.; and
RODDY, J. Serial Passage of Human Tumors in Chick Emb-

4. DUNN, T. B. Normal and Pathological Anatomy of the

5. FOUTS, J. R., and ADAMS, R. H. Drug Metabolism in the

6. GEORGHIU, J. Heterologous Cancer Grafts: The Growth of
Mouse Cancer in Rats. J. Path. & Bact., 29:171–76,
1926.

7. GOMORI, G. Microscopic Histochemistry. Chicago: Uni-

8. HANDLER, A. H. Chemotherapy Studies on Transplanted

9. HARRIS, J. J. Differences in the Behavior of Two Human
Tumors Grown on the Chorioallantois of the Chick Em-


11. HAUSCHKA, T. S.; MITCHELL, J. T.; and NIEDERPRUIM,
D. J. A Reliable Frozen Tissue Bank: Viability and Stability
of 5 Neoplastic and Normal Cell Types after Prolonged

12. KLEIBER, M. Body Size and Metabolic Rate. Physiol.

13. KLENZ, G. Variation and Selection in Tumor Cell Popula-

14. KOSSON, R., and GALLAGHER, F. W. Growth of Human

15. KREMENTZ, E. T., and KOHAME, G. M. HC 305: A Trans-
plantable Human Glioblastoma: Behavior and Response to

16. KUTNER, L. J., and SOUTHAM, C. M. Growth of Human


18. MEKKER, W.; CONDIE, R.; WEINER, D.; VARGO, R. L.; and

19. MULLAY, A. S., and PRICE, E. D., JR. The Histopathology
and Biochemistry of Rats Bearing an Adrenocortical Tu-


21. OLD, L. J.; BURKE, E. A.; and STOCKERT, E. The Role of the Reticuloen-
thelial System in the Host Reaction to Neoplasia. Cancer

22. PATTI, J., and MOORE, A. E., JR. The Histopathology
and Biochemistry of Rats Bearing an Adrenocortical Tu-

23. ROODS, J. Serial Passage of Human Tumors in Chick Em-
byos 180 with Progression to Death of Hosts. Cancer Res-
search, 3:34, 1959.

24. ROSENFELD, K. H.; AXELHAD, A. A.; SIMINOVITCH, L.; Mc-
CULLOCH, E. A.; and PARKER, R. C. The Origin of Altered
Cell Lines from Mouse, Monkey and Man, as Indicated by
Chromosome and Transplantation Studies. Canad. Cancer

25. SIMONSEN, M.; ENGELBHETH-HOLM, J.; JENSEN, E.; and
POULSEN, J. A Study of the Graft—vs.—Host Relation in

26. SIMONSEN, M. The Impact on the Developing Embryo and

27. ROTHFELS, K. H.; AXELHAD, A. A.; SIMINOVITCH, L.; Mc-
CULLOCH, E. A.; and PARKER, R. C. The Origin of Altered
Cell Lines from Mouse, Monkey and Man, as Indicated by
Chromosome and Transplantation Studies. Canad. Cancer

28. SCHRECK, R. Permanent and Transient (Fortuitous) Vari-
ation of the Growth Components of Transplantable Rat

29. SIMONSEN, M. The Impact on the Developing Embryo and

30. SIMONSEN, M.; ENGELBHETH-HOLM, J.; JENSEN, E.; and
POULSEN, J. A Study of the Graft—vs.—Host Relation in
Transplantation to Embryos, FI Hybrids and Irradiated

31. SIKKIND, G. W.; LEONARD, L.; and THOMAS, L. The Run-


---

**FIG. 1.**—Section of liver from suckling mouse (12 days old) not bearing a tumor. Formalin fixation. Histochemical demonstration of acid phosphatase activity (Gomori) in the liver. Mag. X100.

**FIG. 2.**—Section of liver from suckling mouse (12 days old) bearing a growing H.Ep. #8 transplant. Tumor was inoculated when animal was less than 24 hours old. Formalin fixation. Histochemical demonstration of acid phosphatase activity (Gomori) in the liver. Mag. X100.
The Newborn Swiss Mouse as a Host for a Human Epidermoid Carcinoma (H.Ep. #3): Transplantation and Chemotherapy Data

Philip C. Merker, Matthew Bowie and Pauline Anido


Updated version  Access the most recent version of this article at: http://cancerres.aacrjournals.org/content/22/3/352

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.