layer facing that vector, pipetting the cell suspension into the membrane holder, and operating the centrifuge at 50*g* for 15 minutes or at 500*g* for 10 minutes. In general, it is possible to graft stage 2 membranes onto guinea pigs less than 4 hours after harvesting the basal cells from the animals. Promptness of wound closure following injury, an essential component of our design, is thereby achieved. The time required for the seeding step can probably be shortened further.

Following in vitro seeding with uncultured autologous basal cells, stage 2 membranes can be grafted onto full-thickness skin wounds. In the sterile environment of the wound closure, the basal cells rapidly reach confluence, and in less than 14 days form sheets of keratinized epidermis between the top and bottom layers of the membrane (Fig. 3, a and b). Repeated observations have left no doubt that the neoepidermal sheet nucleates and grows from the seeded basal cells rather than originating at the wound edge. Neodermal tissue synthesis also occurs in these seeded membranes and there is no evidence of conventional scar formation. New and apparently functional skin is generated in less than 4 weeks.

The polymeric membranes have also been used successfully on humans. After primary excision of dead tissue (*13*), burn victims received stage 1 membranes as large as 15 by 25 cm (*14*). After being placed on the wound bed, the grafts were carefully sutured under slight tension, avoiding wrinkling of the thin membrane. The subjects, 5- to 60-year-old males and females, received grafts over more than 50 percent of their body surface area. No immunosuppression was employed. Graft take approached 100 percent, providing continuous physiological closure without infection or rejection. Whenever the graft was next to intact epidermis the epidermal edge migrated between the two layers of the membrane over a distance of a few millimeters. Up to 46 days later, the silicone layer was removed from the vascularized artificial dermis and the wound was closed with a thin (0.1 mm) autoepidermal graft. Neither the donor site nor the grafted area showed significant contraction or scarring (*14*). Following removal of split-thickness (0.25 to 0.375 mm) autografts, which include a fraction of the dermis, the donor sites are usually significantly scarred.

We conclude that the noncellular polymeric membrane (stage 1) performs at a level superior to that attained with pig skin or cadaver skin. In fact, when eventually covered with thin autoepidermal grafts, stage 1 membranes appear to equal the autograft in clinical performance. Furthermore, stage 2 grafts provide a means for closing the largest full-thickness skin wounds without delay and without requiring autologous epidermal grafts. Perhaps appropriately designed biodegradable templates can be used to regenerate segments of other tissues or organs which have become lost or dysfunctional due to disease or trauma.

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Tumor Metastasis Is Not Due to Adaptation of Cells to a New Organ Environment

Abstract. *Murine B16 melanoma cells were adapted for lung survival and growth by allowing them to attach to Bio-Carrier beads and injecting the beads intravenously into normal mice. The beads lodged mechanically in the microcirculation of the lung. When the melanoma cells had grown into visible tumors from the arrested beads, the tumors were removed and the cells were dispersed, cultured to remove normal cells, and reattached to new beads. The process was repeated nine times. Previously another B16 subline was injected intravenously as a suspension of separate tumor cells. Those cells that survived and colonized the lungs were harvested, cultured, and injected again. This selection process was also repeated nine times. Only the subline that was injected in suspension was more metastatic than the parental line, indicating that metastasis involves selection of preexistent metastatic cells and is not an adaptive process by which all cells gradually acquire the ability to grow at particular organ sites.*

In tumor metastasis, malignant cells spread from primary sites to nearby and distant secondary sites (*1*). Often, the malignant cells spread preferentially to specific secondary organ sites (*2*). This suggests that unique characteristics of these sites may be important in determining patterns of metastasis. As early as the 1880's, Paget (*3*) proposed that both the secondary tumor environment (the "soil") and properties of the metastatic cells (the "seed") were important in the establishment of distant metastases. In an attempt to determine whether tumor cells gradually adapt to particular environments or are selected for their ability to grow at a given site, Klein (*4*) sequentially converted a solid tumor cell line to grow in peritoneal cavity ascites fluid. She found that the ascites-grown tumor cells preferentially spread to lung tissue and proposed that they had existed in the original tumor as a specialized subpopulation. Since Klein's experiments, several investigators have succeeded in obtaining highly metastatic tumor cell lines by sequentially harvesting metastases, culturing their cells in vitro,

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Table 1. Metastatic potential of the three melanoma cell sublines. In the assay to determine metastatic potential, mice were injected intravenously with 5×10^5 living tumor cells and killed 21 days later.

Subline	Number of pulmonary tumor colonies*	Median number	Site of extrapulmonary tumor colonies†
Experiment 1			
B16-F1	0, 0, 1, 4, 9, 11, 20, 22, 31, 32	11	Ovary (2), lymph node (2), liver (1), abdomen (1)
B16-F1A9	0, 0, 2, 3, 6, 14, 20, 24, 29	6	Ovary (1), lymph node (1), abdomen (2), brain (1), adrenal gland (2)
B16-F10	7, 64, 79, 92, 96, 100, 141, 149, 151	96	Ovary (2)
Experiment 2			
B16-F1	0, 3, 6, 8, 8, 22, 31, 32, 39, 41	8	Abdomen (2), brain (1), mesentery (1), adrenal gland (2)
B16-F1A9	0, 0, 5, 10, 11, 12, 13, 14, 14	11	Ovary (1), lymph node (1), abdomen (2), adrenal gland (1)
B16-F10	0, 52, 89, 101, 109, 110, 132, 136, 140	109	None
Experiment 3			
B16-F1	0, 0, 2, 4, 18, 29, 36, 39, 42, 49	18	Abdomen (2), adrenal gland (1), ovary (1), lymph node (4)
B16-F1A9	0, 0, 3, 11, 13, 13, 21, 27, 28	13	Liver (1), abdomen (4), ovary (1)
B16-F10	30, 46, 51, 79, 89, 100, 111, 114, 114	89	Ovary (1)

*Each value is the number of colonies in one mouse. †Each number in parentheses is the total number of animals with extrapulmonary tumors.

and reestablishing the metastases in vivo (*5*).

Fidler and Kripke (*6*) showed that highly metastatic subpopulations of cells exist in tumors before metastasis occurs. They used cell cloning and fluctuation assays to determine the metastatic potential of individual cells in a melanoma tumor, and found that the parental murine B16 melanoma was composed of a mixture of cell subpopulations; different subclones possessed widely different metastatic potentials. The findings of Fidler and Kripke have since been confirmed for several diverse tumor systems (*7*). This heterogeneity is reminiscent of the various sensitivities of malignant cell clones or sublines to drugs (*8*), radiation (*9*), and heat (*10*). Such cells also vary widely with respect to their lectin-binding sites (*11*), immunogenicity (*12*), and antigen content (*13*).

The fact that certain malignant tumors are composed of subpopulations of cells with different metastatic potentials does not prove the absence of adaptive processes in the preferential growth of tumor cells at sites whose environment is different from that of the primary site. Previous attempts to adapt tumor cells to different local environments depended primarily on tumor cell inoculation at sites different from the usual sites of metastasis. For example, Brunson and Nicolson (*14*) attempted to sequentially adapt B16 melanoma cells to brain tissue by direct intracerebral inoculations. After ten repetitions of this procedure, the "adapted" B16 subline was injected intravenously and assayed for its ability to colonize brain tissue. It was found to be no more metastatic than the parental B16 subline.

We performed an adaptation experiment as follows. Early passage B16-F1 melanoma cells were grown and attached to Bio-Carrier beads (diameter, 120 to 180 μm) (*15*). The use of these beads circumvents the possibility of tissue damage caused by direct inoculation with a syringe needle. The beads, which in most cases were completely covered (approximately 50 cells per bead) by melanoma cells (Fig. 1), were injected intravenously into groups of syngenic C57BL/6 mice (30 beads per animal). The beads lodged mechanically in the microcirculation of the lung (*16*). In 13 to 21 days small tumor colonies formed exclusively in the lungs, each colony completely surrounding a bead (Fig. 2). Some of these colonies were harvested and cultured to remove contaminating host cells. This subline was designated B16-F1A1. The procedure was repeated eight times in sequence to obtain subline B16-F1A9, which thus had been adapted nine times for growth in the lung. Another subline (B16-F10) was obtained previously (*5*) by intravenously injecting mice with suspensions of separate tumor cells and harvesting the colonies that grew in the lungs. The procedure was performed nine times in sequence. The parental line and the B16-F1A9 and B16-F10 sublines were then assayed for their ability to colonize the lung after injection in suspension and for their ability to spread to the lung spontaneously after implantation at subcutaneous sites (*17*). Subline B16-F1A9 colonized the lung to the same extent as the original B16-F1 cells; however, subline B16-F10 formed signifi-

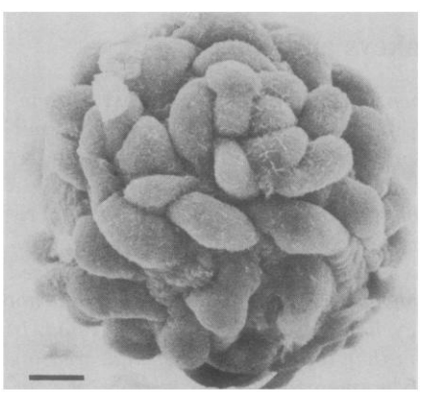

Fig. 1. Scanning electron micrograph of B16-F1A9 cells attached to a Bio-Carrier bead. A sample of the beads with attached cells (*15*) was washed twice in 0.125*M* sodium cacodylate buffer (*p*H 7.3) and fixed in cacodylate buffer containing 1 percent glutaraldehyde and 10 percent formaldehyde for 30 minutes at 22°C. The beads were then rinsed in cacodylate buffer for three 3-minute periods and postfixed in cacodylate buffer containing 2 percent OsO_4 for 20 minutes at 22°C. After three more rinses, the beads were dehydrated in ethanol, transferred to Freon, and critical-point-dried (Bomar model SPC-1500). The beads were coated with gold and palladium under vacuum in a Hummer V (Technics) and examined in an AMR-1000A scanning electron microscope. Scale bar, 10 μm.

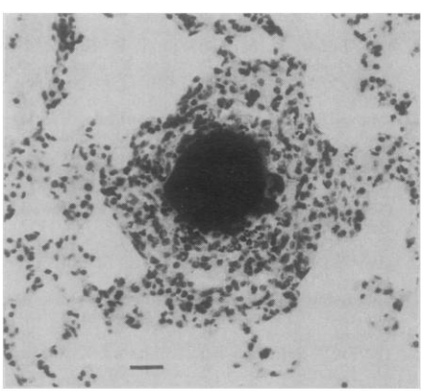

Fig. 2. Photomicrograph of a section of mouse lung 13 days after intravenous injection of B16-F1 melanoma cells attached to Bio-Carrier beads. The melanoma cells have grown by simple expansion. Stain, hematoxylin and eosin; scale bar, 50 μm.

cantly more lung tumor nodules than either B16-F1 or B16-F1A9 (Table 1). Also, in spontaneous metastasis assays (*17*) the adapted subline, B16-F1A9, was no more metastatic than the original B16-F1 subline, indicating that mere growth of B16 cells in the lung is insufficient to produce more metastatic cells. Thus, sequential adaptation of tumor cells to an organ environment through a nondestructive method of cell transplantation yields results similar to those of other procedures. These data do not support an adaptation theory for successful metastatic colonization.

Raz *et al.* (*18*), who utilized an ultraviolet radiation–induced fibrosarcoma, came to the same conclusion. By intravenously injecting fibrosarcoma cell clones with low lung colonization potential, harvesting the few lung tumor nodules that formed, culturing these in vitro, and retesting their lung colonization potentials, Raz *et al.* found that one cycle of tumor cell growth in the lung is not sufficient to increase the potential of a given cell line to form pulmonary metastases. In addition, Raz *et al.* found that the potential of parental fibrosarcoma cells to spread to the lung is enhanced when they are injected intraperitoneally, harvested, and grown in vitro.

Thus, it appears that tumor cells adapted to the lung do not have an increased ability to spread to that site. Metastasis is not an adaptive process; instead, it appears to involve selection of preexistent tumor cells with higher metastatic potential (*1, 6, 7, 14, 18*).

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Stereoisomers of *N*-Allylnormetazocine: Phencyclidine-Like Behavioral Effects in Squirrel Monkeys and Rats

Abstract. *(±)-N-Allylnormetazocine is a benzomorphan opioid with psychotomimetic effects. The pure stereoisomers of this compound, as well as the racemic mixture, were compared to phencyclidine for their behavioral effects on squirrel monkeys and rats trained to discriminate phencyclidine from saline. Dose-response determinations were made for responses to phencyclidine, to a racemic mixture of N-allylnormetazocine, and to the pure levo and dextro isomers of N-allylnormetazocine. In both rats and monkeys, the dextro isomer and the racemic mixture produced dose-dependent responses appropriate for phencyclidine; the levo isomer did not produce the responses appropriate for phencyclidine at any of the doses tested. In both species, the levo isomer was more potent than the dextro isomer in decreasing the rate of responding. Thus racemic N-allylnormetazocine is a mixture of compounds that produce different behavioral effects.*

The pharmacological properties of the dissociative anesthetics, phencyclidine (PCP) and ketamine, appear to overlap those of the psychotomimetic opioids. The effects of PCP on the dog with transected spinal cord (*1*) are similar to the effects of (±)-*N*-allylnormetazocine (SKF 10,047), a benzomorphan opioid considered to be the prototypical agonist of the putative σ opiate receptor (*2*). Rats trained to discriminate PCP from saline generalize the PCP response to a series of structural analogs of PCP and to (±)-*N*-allylnormetazocine but not to some other psychoactive substances (*3*). Rats and pigeons trained to discriminate cyclazocine, another psychotomimetic benzomorphan, from saline generalize the cyclazocine response to PCP, ketamine, and dextrorphan (*4*). Rhesus monkeys generalize their responses to ketamine to (±)-*N*-allylnormetazocine and dextrorphan, but not to cyclazocine nor to the levo (−) isomer of dextrorphan, levorphanol (*5*). Because the dissociative anesthetic–like effects of some opioids

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