approximately 0.1 percent anabaseine (by volume) initially attract more ants to the area. However, the presence of anabaseine then prevents feeding. The ants are attracted to the vicinity of the test solution, touch the treated homogenate with their mouthparts, and then quickly back up. Such behavior, rarely observed at the control homogenates, might be interpreted as a mild alarm response and suggests that anabaseine is a gustatory repellant when contacted orally or ingested.

Nitrogenous compounds released from the venom gland of myrmicine ants have been demonstrated to elicit trail-following behavior in *Atta* (*16, 17*) and *Monomorium* (*2*). Anabaseine, tested according to the method of Moser and Blum (*18*), elicited no such behavior in *A. fulva*. Although nitrogenous compounds are not common exocrine products in ants (*19*), several have been identified in the poison glands of myrmicine genera. We believe that the poison gland secretions of myrmicine ants will prove to be a rich source of such alkaloids.

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References and Notes

1. J. G. MacConnell, M. S. Blum, H. M. Fales, *Science* **168**, 840 (1970); *Tetrahedron* **26**, 1129 (1971).
2. D. J. Pedder, H. M. Fales, T. Jaouni, M. Blum, J. MacConnell, R. M. Crewe, *Tetrahedron* **32**, 2275 (1976); T. H. Jones, M. S. Blum, H. M. Fales, *Tetrahedron Lett.* (1979), p. 1031; M. S. Blum, T. H. Jones, B. Hölldobler, H. M. Fales, T. Jaouni, *Naturwissenschaften* **67**, 145 (1980).
3. F. J. Ritter, I. E. M. Rotgans, E. Talman, P. E. J. Verwiel, F. Stein, *Experientia* **29**, 530 (1973); E. Talman, F. J. Ritter, P. E. J. Verwiel, in *Proceedings of the International Symposium on Mass Spectrometry in Biochemistry and Medicine, Milan*, A. Frigerio and N. Castagnoli, Eds. (Raven, New York, 1974), p. 197.
4. *Aphaenogaster fulva* (Roger) and *A. tennesseensis* (Mayr) were collected from rotten logs on the Howard University property in Beltsville, Md., and on the Marine Training Base in Quantico, Va.
5. We used a Finnigan 3200 combined gas chromatograph–mass spectrometer with 1.6-m 3 percent OV-17 and 10 percent SP-1000 columns on Supelcoport 60/80, programmed at 10°C per minute from 40°C to 200°C (300°C for OV-17).
6. Proton magnetic resonance spectra were obtained with a Varian 220 Fourier-transform spectrometer at the National Institutes of Health. Subsequent analyses were performed with the NT-200 instrument at Howard University.
7. L. M. Jackman and S. Sternhell, in *Application of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry* (Pergamon, Oxford, ed. 2, 1969), pp. 211 and 307.
8. L. F. Fieser and M. Fieser, *Reagents for Organic Synthesis* (Wiley, New York, 1967), p. 125.
9. Manufactured by Aldrich Chemical Co., Milwaukee.
10. E. Spath and L. Mamoli, *Ber Dtsch. Chem. Ges.* **69**, 1083 (1936).
11. H. Kamimura and I. Yamamoto, *Agric. Biol. Chem.* **27**, 450 (1963).
12. A. M. Duffield, H. Budzikiewicz, C. Djerassi, *J. Am. Chem. Soc.* **87**, 2926 (1965).
13. W. R. Kem, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **28**, 610 (1969); W. R. Kem, B. C. Abbott, R. M. Coates, *Toxicon* **9**, 15 (1971); W. R. Kem, *ibid.*, p. 23.
14. Five microliters of a 0.5 percent (by volume) solution of anabaseine in methylene chloride was applied to an 8-mm-diameter Whatman filter paper disk. The methylene chloride was allowed to evaporate for 30 seconds before testing was begun. Five microliters of methylene chloride was applied to an 8-mm-diameter Whatman filter-papeated six times.
15. Mealworms were homogenized in 0.1 ml of water. The test solution consisted of 10 μl of mealworm homogenate and 5 μl of anabaseine standard (*14*). Control solutions consisted of 10 μl of mealworm homogenate and 5 μl of methylene chloride. Each solution was placed on a slide and allowed to evaporate for 30 seconds before testing was begun.
16. J. H. Tumlinson, R. M. Silverstein, J. C. Moser, R. G. Brownlee, J. M. Ruth, *Nature (London)* **234**, 348 (1971); H. J. Cross *et al.*, *J. Chem. Ecol.* **5**, 187 (1979).
17. R. G. Riley, R. M. Silverstein, B. Carroll, *J. Insect Physiol.* **20**, 651 (1974).
18. J. C. Moser and M. S. Blum, *Science* **140**, 1228 (1963).
19. M. S. Blum and H. R. Hermann, in *Handbook of Experimental Pharmacology*, vol. 48: *Arthropod Venoms*, S. Bettini, Ed. (Springer, Berlin), pp. 805–809.
20. We thank Dr. David Smith for identifying the specimens.

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Living Tissue Formed in vitro and Accepted as Skin-Equivalent Tissue of Full Thickness

Abstract. *Living skin-equivalent grafts consisting of fibroblasts cast in collagen lattices and seeded with epidermal cells were successfully grafted onto the donors of the cells. The grafts were vascularized, did not evoke a homograft reaction, inhibited wound contraction, filled the wound space, and persisted.*

We previously showed that a collagen lattice seeded with skin fibroblasts contracts into a tissue and that this phenomenon is dependent on the concentration of cells and protein (*1*). We also reported that suspensions of epidermal cells, applied to the contracted lattices in vitro, cover them rapidly and differentiate thereon (*2*). The epidermis becomes multilayered and undergoes keratinization. We now find that such living tissue complexes, fabricated in vitro, can be grafted onto the animals that provide the cells. These grafts become well vascularized and are incorporated into the host much as are autografts of full thickness. Further, the incorporated graft inhibits wound contraction so that the final area occupied by the graft is essentially unchanged.

To prepare a skin-equivalent graft, fibroblasts are isolated from a skin biopsy from a rat donor, cultured, harvested, and used to populate a collagen lattice in vitro (*1*). The cells used to populate the lattice are taken from the potential graft recipient. The proliferation of cells in vitro permits the fabrication of a tissue of any required size. Five days to 1 week later an epidermal biopsy is taken from the back of the same animal with a dermatome, dissociated, and applied as a cell suspension to the contracted, dermal-equivalent lattice containing the fibroblasts. The graft is fitted into an open skin wound made on the back of the donor rat, sutured in place, and bandaged. For long-term grafts the bandages are removed after 10 days.

We applied a total of 52 grafts measuring 6 to 8 cm^2 each; no rejections were observed. The oldest graft was retained for 68 days, at which time it was excised for examination. In a more recent series of experiments, the oldest grafts have been in place for 13 months at this writing (*3*). To ascertain the degree to which the host is sensitized by the graft, second grafts were given to animals that had received grafts previously. Cell stocks from original biopsies were used again to make up the lattices; fresh biopsies were made to obtain the epidermal cells. We found that the rats were not sensitized by the first graft. For example, graft 29 was in place for 43 days, at which time it was removed. Later, graft 29′ was applied and left in place for an additional 35 days, giving a total of 78 days of acceptance by the same animal.

About 80 percent of the grafts retained their original size and shape, thus inhibiting wound contraction (Fig. 1, a and b). The grafts were hairless and smooth (Fig. 1c) and, therefore, readily distinguishable from the surrounding tissue. Examined from beneath, the grafts, like autografts of full thickness, appeared somewhat thinner than the surrounding tissue. Not all the grafts took well; about 20 percent became partly dislodged and showed signs of inflammation where contact with adjacent skin was interrupted. We attribute these failures to inadequate immobilization of the grafts during the early period after grafting.

Four days after epidermal cells are seeded onto the lattice, the lattice can be observed to be partly covered with poorly organized epidermis. There is little evidence of a coherent basal layer, and the epidermal cells are irregularly distributed. After 5 weeks, however, a fairly regular basal layer is present with

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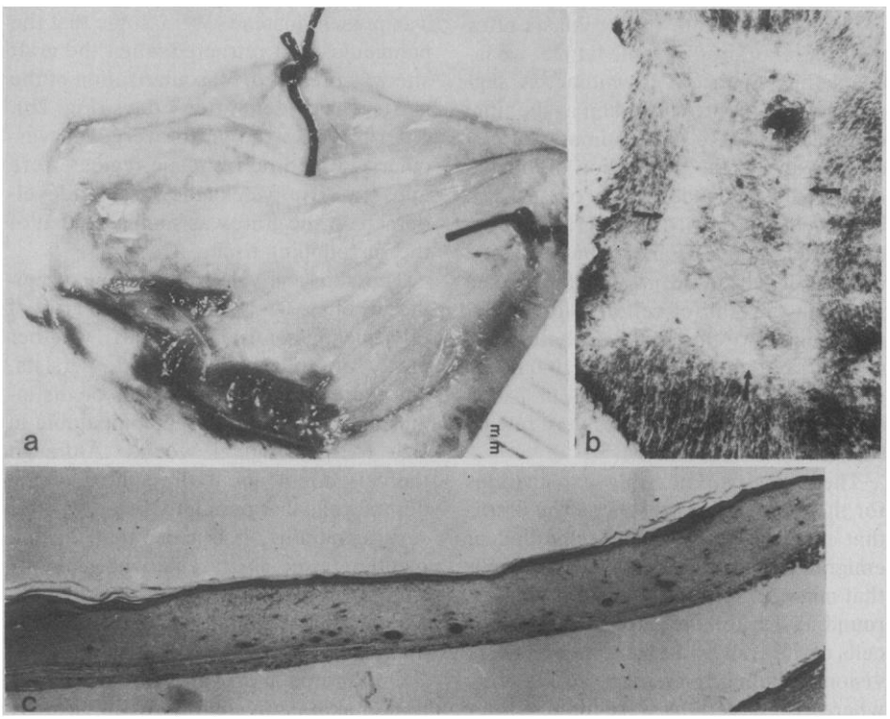

Fig. 1. Gross appearance of grafts (a) 4 days and (b) 50 days after being applied to male Sprague-Dawley rats. (c) Photomicrograph of a section of a 50-day-old graft, showing the absence of hair follicles and sebaceous glands. To prepare a skin-equivalent graft, a small area on the lower back of an animal anesthetized with ether is shaved and washed with 70 percent ethanol. A 0.5-cm square of skin is removed, cut up into 0.1-cm cubes, and washed six times with sterile medium containing gentamicin and Fungizone. The cubes are deposited on petri dishes and allowed to adhere. Eagle's minimum essential medium (Dulbecco's modification) containing 10 percent fetal calf serum and antibiotics is added to a level just covering the explants. The cultures are incubated for 1 week at 37°C with 5 percent CO_2 and the medium is replaced twice. After 1 week enough cells have grown out from the explants for the explants to be discarded. The fibroblasts are allowed to grow to confluency and the cells are placed in 25-cm² Falcon flasks with 6.0 ml of fresh medium. The medium is changed twice a week. At the time of the second change, if labeled cells are required, [^{3}H]thymidine (0.2 μCi/ml) is added and the cells are incubated for 24 hours. (The labeled medium is then discarded and replaced by fresh unlabeled medium.) The cells are combined with collagen and other components (*1*) to form a lattice. The donor rat is anesthetized and a dermatome is used to remove a small area of its epidermis, which is washed in McKeehan's solution 1 (*6*) and dissociated in solution 1 with trypsin (0.25 percent) and EDTA (*p*H 7.6). The epidermal cells are then seeded onto the lattice. After a minimum of 2 days the graft is fitted into an open wound on the back of the donor rat and sutured into place with two to four sutures. A Telfa pad soaked in culture medium is placed over the area and secured with one suture. The rat's entire back is then covered with Elastoplast, and the animal is placed alone in a cage.

overlying strata of differentiating cells. No evidence of secondary epidermal derivatives is seen.

After 9 weeks the epidermis is very well developed. The improved organization of the basal layer is seen in the uniform alignment of the constituent cells. Keratohyalin granules are present throughout the granulosum and a mat of keratinized cells overlies the entire epidermal surface. The graft epidermis is hyperplastic and still of variable thickness, in places being two times thicker than normal epidermis.

Pegs of epidermal cells penetrating the dermis at various distances from one another were seen in 7-week-old grafts. Also, the host's epidermis was thickened for a distance of several millimeters adjacent to the graft.

There is some question about the origin of the epidermal cells that finally cover the graft. We do not know whether the epidermis surrounding the graft contributes cells or whether the applied cells provide all of the final epidermal covering. It is certain that the applied cells are present in the graft quite early and that in vitro they quickly cover the substrate and undergo normal keratinization (*2*). Recent experiments with large grafts (8 by 10 cm) that persist and do not desiccate suggest that the applied epidermis is retained.

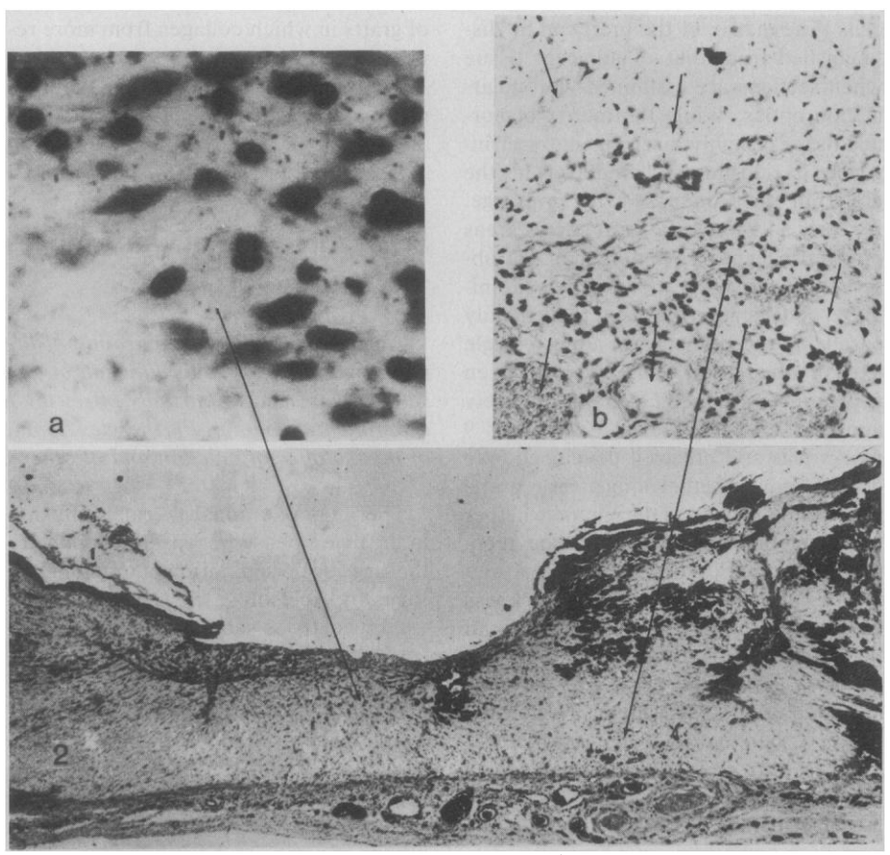

Fig. 2. Autoradiograph of a section of a 1-week-old graft showing labeled fibroblasts (×11). (a) Enlargement of the area to which long arrow points. Heavily labeled nuclei are black and unlabeled nuclei are gray (×280). (b) Enlargement showing evidence of vascularization (small arrows). Autoradiographs were made by dipping histological sections in NTB emulsion (Eastman). After exposures of 1 to 3 weeks, slides were developed and examined by light microscopy.

To follow the fate of fibroblasts after their incorporation into the lattice, we labeled them with [^{3}H]thymidine. A segment of each graft was taken at the time of implantation so that estimates of cell density and of the proportion of labeled cells could be made at the outset. Labeled cells were still seen in grafts recovered after 7 days (Fig. 2), but the total number of cells in the graft had increased greatly as a result of cell division or cell migration into the graft or both. The number of labeled cells in 10-day-old grafts was similiar to that in 6- or 7-day-old grafts, but after 5 weeks very few labeled cells were detected.

There are two possible explanations for their decreased presence. The first is that many of the original cells died or emigrated and were replaced by cells that migrated into the graft from the surrounding tissue; the second is that the cells of the graft underwent numerous divisions, diluting the isotope to the point where disintegrations were too few to be detected after a 3-week exposure.

The organization of the graft in the rat superficially resembles that of normal surrounding tissue, except that hair follicles and sebaceous glands are absent. Fibroblasts are distributed throughout, with intercellular matrix separating the cells. The matrix of the graft can be distinguished from that of adjacent tissue when sections are examined with polarization optics. While the matrix of normal tissue is highly birefringent, birefringence is substantially reduced in the graft but does increase with graft age. As early as 1 week the graft shows areas of birefringence, a phenomenon not observed so early in granulation tissue. Initially, collagen fibrils appear to lie mainly parallel to the skin surface along a single axis of orientation. The typical woven appearance of the collagen mat is barely apparent after 7 weeks. Even after 9 weeks it is still not well developed. We do not know whether longer-term grafts will exhibit a normal dermis or whether its development depends on the technique of lattice casting.

After 10 weeks the average thickness of the graft dermis measured about half that of the adjacent dermis, which was about 1.3 mm. The dermis of the graft appeared much less spongy and more compact than that of adjacent skin; however, in the zone of transition between them, the dermis was spongier. Also, the graft dermis was more birefringent in the transition zone than anywhere else.

In most of the grafts we examined, the panniculus layer normally found underlying the dermis was absent; loose connective tissue with many adipose cells was present instead. We assume that the panniculus had retracted when the graft site was prepared. Vascularization of the grafts was evident after 7 days (Fig. 2b). After 5 weeks the grafts were well vascularized, and no ischemic regions were apparent. By 7 weeks the vascular development of the graft was similiar to that of the surrounding tissue.

The skin-equivalent graft, unlike epidermis alone (*4*, *5*), acts as a skin graft of full thickness. And the graft, whether partly or entirely remodeled, persists, since after many months it can be distinguished as a hairless area comparable in size to the original wound. Although there is doubt about the source of the dermal cells that populate the grafts after several months, it is clear that the implanted tissue is vascularized, inhibits wound contraction, fills the original wound, and eventually resembles skin in many respects.

Our approach provides for the fabrication of skin-equivalent tissue in virtually unlimited amounts from a small biopsy, since it depends mainly on replication in vitro of the required number of dermal and epidermal cells. Although we have successfully used rat collagen as a lattice for grafts composed of guinea pig cells, we have not yet tested the acceptability of grafts in which collagen from more remote species is used.

We think that the most crucial prerequisite for graft acceptance is contraction and conditioning of the lattice by cells from the individual who will receive the graft. The practical uses of the skin-equivalent graft are obvious, particularly since the substance can be cast into virtually any shape. However, its potential for reducing distortion and functional impairment in human injuries requiring skin replacement remains to be established.

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References and Notes

1. E. Bell, B. Ivarsson, C. Merrill, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 1274 (1979).
2. E. Bell, C. Merrill, D. Solomon, *J. Cell Biol.* **83**, 398 (Abstr.) (1979).
3. S. Sher, B. Hull, R. Sarber, E. Bell, in preparation.
4. R. E. Billingham and P. B. Medawar, *J. Anat.* **89**, 114 (1955).
5. R. E. Billingham and P. S. Russell, *Ann. Surg.* **144**, 961 (1956).
6. W. L. McKeehan, *Cell Biol. Int. Rep.* **1**, 335 (1977).
7. We thank R. Trelstad for advice and consultations on graft histology. This study was supported in part by grants from Flow Laboratories to E.B. and by PHS grant GN25561 to H.P.E.

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Separation of Calcium Isotopes by Liquid Phase Thermal Diffusion

Abstract. *Significant separation of the isotopes of calcium was obtained by thermogravitational thermal diffusion of an aqueous calcium nitrate solution. A flow of solvent was used to partially offset the large solute-solvent separation effect in the experimental column. Further development of this technique may lead to separation of the isotopes of calcium and of other elements on a practical scale.*

Thermogravitational thermal diffusion in the liquid phase (*1*) was first applied to the large-scale separation of uranium isotopes by Abelson *et al.* (*2*). It has been used recently to separate practical quantities of sulfur (*3*), chlorine (*4*), and bromine (*5*) isotopes. Many elements, however, do not have stable, low-molecular-weight, liquid compounds in a temperature range suitable for thermal diffusion separation. In principle, it is possible to separate these elemental systems as solutions of some solid compound in a suitable solvent. Normally, the separation of solute from solvent is much greater than the separation of isotopic species; therefore, in an efficient thermal diffusion column nearly pure solvent accumulates at one end of the system and essentially all of the solute concentrates at the other end.

For nonisotopic systems, Korsching (*6*) demonstrated that the solvent-solute separation could be suppressed by imposing a net flow of solvent through the separation column. It can be shown (*7*) that in a dilute solution the solvent flow does not affect the separation of the components of the solute. In this report we describe the separation of calcium isotopes by the Korsching technique and derive some conditions necessary for its successful application.

If we consider the system at first as a

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