pletely normal fashion to blastocyst, when they may be reimplanted into the uterus of a foster mother for further development. Blastomeres of naked eggs during all stages of cleavage tend to remain in contact with their neighbors, though they may be separated with versene (*6*).

The mouse blastocyst exhibits a striking spontaneous "hatching" out of the zona pellucida in vitro (*9*). One or more spherical projections, each with the wall composed of a single-cell, extend from the thinly stretched trophoblast through what appear to be small pores in the zona pellucida. The remainder of the blastocyst then emerges through a widened crack, and the zona is left behind but does not disappear. This is also presumed to occur in vivo prior to implantation. The possibility suggests itself that blastocyst emergence might result simply from mechanical pressure against the zona as the internal cavity expands with fluid. Eggs were therefore observed in culture after the membrane had been stripped at an earlier stage. Lobes were still "extruded" from the surface in a simulation of hatching. It was also noted that the zona of the morula is more easily ruptured by pipetting than in earlier stages. These data suggest that the zona becomes modified in some way during late cleavage and that some active process is involved in the passage of the blastocyst out of the zona.

The surfaces of eggs from which the zona pellucida has been removed are considerably stickier than they are when the membrane is still present, and the eggs tend to adhere to each other. The fusion is greatly accelerated at 37°C, and this fact has served as the basis of a method for synthesizing genetic mosaics (*4*, *8*). Accidental loss of the zona pellucida when two or more eggs are present together in vivo could therefore lead to formation of mosaic individuals. An example of this in the human might be the XX/XY case described recently by Gartler *et al.* (*10*).

BEATRICE MINTZ
Institute for Cancer Research, Philadelphia 11, Pennsylvania

References and Notes

1. C. R. Austin and J. E. Lovelock, *Exptl. Cell Res.* **15**, 260 (1958).
2. A. W. H. Braden, *Australian J. Sci. Res. Ser. B* (now *Australian J. Biol. Sci.*) **5**, 460 (1952); B. V. Hall, *Proc. Soc. Exptl. Biol. Med.* **32**, 747 (1935); M. Smithberg, *Anat. Record* **117**, 554 (1953); M. C. Chang and D. M. Hunt, *Exptl. Cell Res.* **11**, 497 (1956).
3. Calbiochem, B grade.
4. B. Mintz, *Am. Zoologist* **2**, 432 (1962). Supported by U.S. Public Health Service grant C-5201 (C-2). Use of this enzyme for removal of the zona pellucida was first described at a meeting of the Tissue Culture Association in Washington, D.C. (29 May 1962), and has since been adopted by a number of laboratories.
5. M. Nomoto and Y. Narahashi, *J. Biochem. Tokyo* **46**, 653 (1959); K. Satake, S. Kurioka, T. Neyasaki, *ibid.* **50**, 95 (1961).
6. B. Mintz, in preparation.
7. A. W. H. Braden, C. R. Austin, H. A. David, *Australian J. Biol. Sci.* **7**, 391 (1954).
8. B. Mintz, *Am. Zoologist*, in press.
9. W. K. Whitten, *J. Endocrinol.* **16**, 80 (1957).
10. S. M. Gartler, S. H. Waxman, E. Giblett, *Proc. Natl. Acad. Sci. U.S.* **48**, 332 (1962).

18 October 1962

Totipotency of Cells from Fruit Pericarp Tissue in vitro

Abstract. Callus derived from avocado fruit pericarp grown in vitro for several generations developed roots with stele, endodermis, cortex, epidermis, and root cap. No correlation between environment and root production was demonstrated.

Plant tissue cultures generally isolated from vascular cambium, such as carrot root phloem, or from stem or root pith have been widely used for morphogenetic studies.

Attention has been given recently to basic requirements for the induction of proliferation of specific fruit-tissue explants in vitro. Pericarp and mesocarp tissues from such diverse fruit types as apple (*1*), citron (*2*), peach (*3*), pear, quince (*4*), and tropical fruits such as avocado, banana, and cherimoya (*5*) have been shown capable of cell division and enlargement when provided with a suitable nutrient medium. However, most of these fruit tissues have not been established in continuous culture. Exceptions thus far are apple (*1*), juice vesicle stalks from lemon (*6*), the pericarp of citron (*Citrus medica* L.), and the pericarp of avocado (*Persea americana* Mill.). The latter is of particular concern in this report because of the high degree of differentiation that occurred over a period of 1 year, resulting in the appearance of roots. That such a tissue as fruit pericarp should form roots is significant in demonstrating the totipotency of the tissue.

The explants used in these studies were obtained from pericarp of a nearly mature Hass avocado fruit. Tissue disks 8 mm in diameter and 2 mm thick were cut, under aseptic conditions, from a surface-sterilized fruit by means of a stainless steel slicer and borer. The disks were placed in screw-top vials containing Nitsch's basic solution (*7*) modified by the use of iron in the form of chelate No. 138 HFe (Geigy Company) (0.5 part per million) instead of ferric citrate, and by the addition of indoleacetic acid (10 parts per million) and 0.8 percent agar. The cultures were kept in a storage box, with no particular control of temperature or light, and allowed to grow for 6 weeks. Newly formed callus was then removed and cut into several pieces and subcultured on fresh medium. Cultures thus established were increased through routine subtransfers every 6 to 8 weeks. Anatomical observations were made on fresh and prepared materials.

The explants grew actively in the medium in the first 4 weeks; their fresh weight nearly doubled. Cell proliferation and cell enlargement occurred, forming abundant callus. Active proliferation was observable as white spongy growth which later developed into more compact callus masses. These became enlarged brown, nodular, corked areas. Friability was not observed to the degree attained by us in citron callus (*8*) or reported by other workers on pea (*9*), spruce (*10*), and cactus (*11*). Visible surface proliferation ceased after 4 to 6 weeks, although the cultures remained viable.

After 1 year, roots appeared in several of the subcultures, which were four to six generations removed from the original explants (Fig. 1*A*). No correlation could be made between conditions of the environment and the high degree of differentiation. Limited experiments in which concentrations of indoleacetic acid, kinetin, gibberellin, or thiamine were varied in the medium did not induce root development in comparable materials.

The roots penetrated into the agar or appeared on the upper exposed callus surface. The longest root was 4 mm long and 0.5 mm in diameter at the time it was collected. The roots were typically cylindrical, constricted somewhat at the point of emergence. A definite root cap developed around the ellipsoidal tip (Fig. 1*B*). Morphologically, the roots consisted of a central primary stele in tetrarch arrangement, a well-defined endodermis, and a cortex of starch-filled parenchyma cells delimited by a distinct epidermis with thick, suberized outer cell walls (Fig. 1*C*).

Microscopic observations of 9- to

14-week-old tissue masses revealed differentiation within the callus similar to that reported by other workers in callus from various tissues of vegetative origin (*12–14*), except that the tracheid nests in the materials of our study were not completely enclosed by a ring of cambium-like cells. The islands of differentiating tissues formed, in some instances, long finger-like projections through the callus mass (Fig. 1, *D* and *E*). In the primordia from which roots arose, ground parenchyma lost its loose, disorganized appearance and became compact, with lignified, isodiametric cells full of compound starch grains. The associated tracheids in this tissue showed secondary thickening and pitted walls. Newly formed roots generally could be traced to isolated nests of cells of the latter type. Surrounding these discrete, nodular, tracheid nests were dying or dead crushed cells containing much tanniferous material. The remainder of the callus mass was more or less disorganized. Cells with darkly staining contents, similar to those found in vivo, frequently were present in rows, sometimes extending into the compact areas. Other cells, also in raylike arrangement, contained starch. Crystals were not common, but some, mostly acicular, did occur in unlignified areas. Outer regions of the callus frequently consisted of typical wound periderm.

Vascular patterns of avocado roots in vivo are hexarch or octarch in arrangement, with a central pith. Very small or weak roots sometimes show tetrarch arrangement. Only the tetrarch pattern, with no pith, was observed in vitro. Changes in the vascular patterns of roots have been related to auxin concentrations in the media (*13, 15*). Pea roots grown at high auxin concentrations exhibit a complex vascular pattern, whereas transfer of the roots to medium with low concentrations of auxin results in a reduction of complexity.

The factors leading to the formation of roots from avocado pericarp have not been fully evaluated. Possibly auxin concentrations in relation to other constituents of the medium, as demonstrated for root differentiation in pea (*9, 13*), tobacco pith (*16*), and carrot (*14*), may be involved. The age of the original callus and of the subcultures may also be factors, or a relationship between polyploidization and root differentiation may exist (*17*). That callus cells derived from such a tissue as fruit pericarp should give rise to roots is highly significant. The study described in this report is, we believe, the first to demonstrate totipotency of cells from organs other than those of vegetative origin in higher plants.

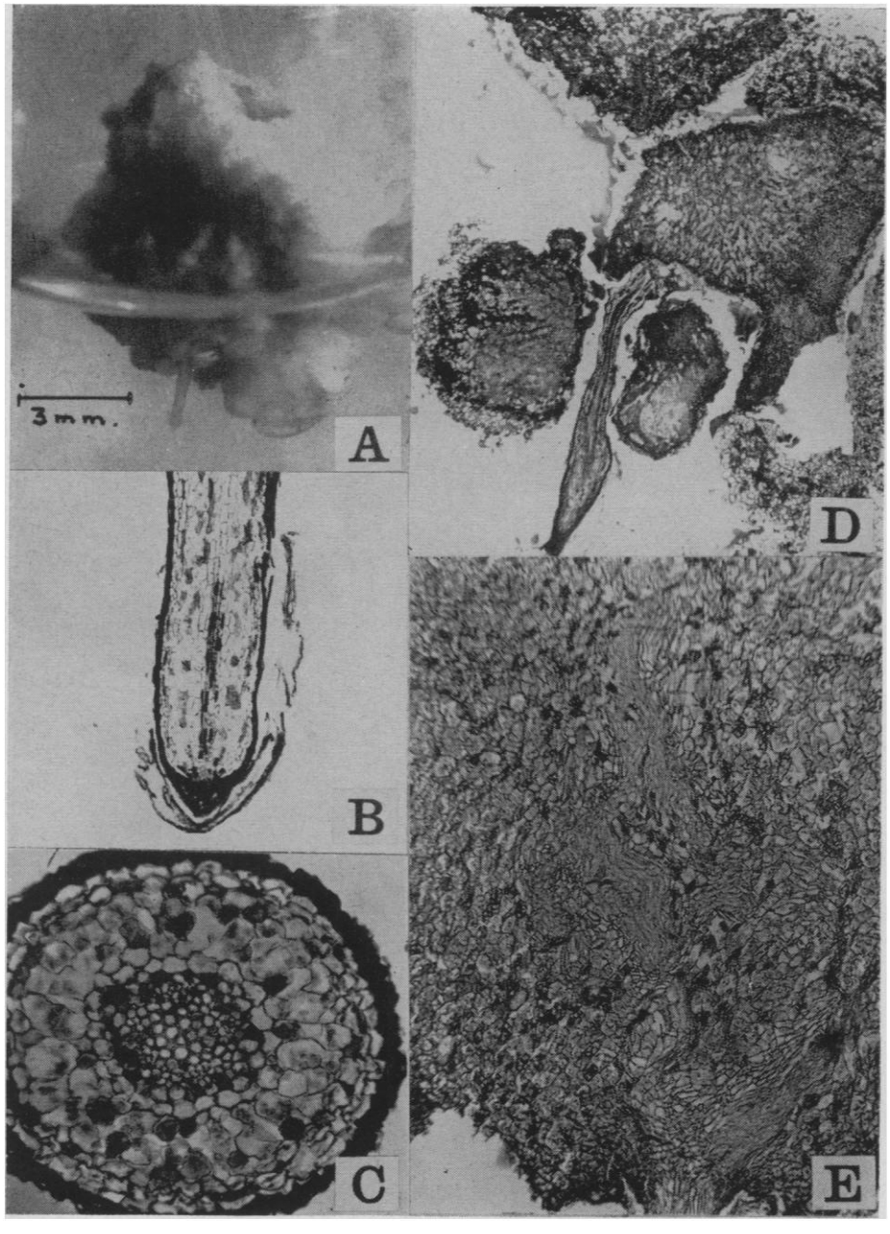

Fig. 1. *A*, Avocado pericarp callus in vitro with emerging root. *B*, Longitudinal section through root, showing root cap. *C*, Transverse section through root, showing stele, endodermis, cortex, and epidermis. *D*, Origin of root in callus. *E*, Detail of differentiated callus, showing tracheid nests from which roots arise.

C. A. Schroeder
E. Kay
Lily H. Davis
Department of Botany,
University of California, Los Angeles

References

1. D. S. Letham, *Nature* **182**, 473 (1958).
2. C. A. Schroeder and C. Spector, *Science* **126**, 701 (1957).
→ N. F. Sommer, M. V. Bradley, M. T. Creasy, *ibid.* **136**, 264 (1962).
4. D. S. Letham, *Nature* **188**, 425 (1960).
5. C. A. Schroeder, *Botan. Gaz.* **122**, 198 (1961).
→ H. A. Kordan, *Bull. Torrey Botan. Club* **89**, 49 (1962).
→ J. P. Nitsch, *Am. J. Botany* **38**, 566 (1951).
8. C. A. Schroeder, E. Kay, L. H. Davis, unpublished data.
→ J. G. Torrey and Y. Shigemura, *Am. J. Botany* **44**, 334 (1957).
10. J. Reinert, *Science* **123**, 457 (1956).
11. C. E. Steinhart, *ibid.* **137**, 545 (1962).
12. R. J. Gautheret, *La Culture des Tissus Végétaux* (Masson, Paris, 1959).
→ H. P. Sorokin, S. N. Mathur, K. V. Thimann, *Am. J. Botany* **49**, 444 (1962).
→ F. C. Steward, M. O. Mapes, K. Mears, *ibid.* **45**, 705 (1958).
→ J. G. Torrey, *ibid.* **44**, 859 (1957).
16. F. Skoog, *Brookhaven Symp. Biol.* **6** (1954).
17. J. G. Torrey, in *Cell, Organism and Milieu* (Ronald, New York, 1958).

26 September 1962