Morphogenetic Responses of Cultured Totipotent Cells of Carrot (*Daucus carota* var. *carota*) at Zero Gravity

Abstract. *An experiment designed to test whether embryos capable of developing from isolated somatic carrot cells could do so under conditions of weightlessness in space was performed aboard the unmanned Soviet biosatellite Kosmos 782 under the auspices of the joint United States–Soviet Biological Satellite Mission. Space flight and weightlessness seem to have had no adverse effects on the induction of embryoids or on the development of their organs. A portion of the crop of carrot plantlets originated in space and grown to maturity were not morphologically different from controls.*

Plants respond during their development to a wide range of known environmental factors including gravity. On-earth and short-term space studies have been limited to tropistic responses of mature or preformed organs. No studies have explored gravitational effects before the formation of these organ systems. The near-zero-gravity space environment introduces an entirely new set of conditions which can be used to study this relationship. We report here the results of an experiment carried out to test whether embryogenesis in higher plants is in any way dependent on or conditioned by the 1*g* gravitational stimulus to which organisms have been inevitably and universally exposed during their evolution. It was possible to do this, not with isolated fertilized eggs, which are very difficult to isolate and grow in quantity, but with somatic cells, which can be cultured aseptically in large numbers and allowed to grow and develop in a somatic embryogenesis which simulates zygotic embryogenesis. For some years it has been possible to bring somatic cells of carrot in liquid through the definitive stages of embryogenesis to the formation of embryoids and small plantlets (*1*). In fact, in this way it is now far more feasible to grow embryos and raise them to maturity from somatic cells than to do so from fertilized eggs from embryo sacs.

The normal conditions under which carrot somatic embryogenesis has been practiced routinely in this laboratory have been modified and rendered compatible with space flight by using a semi-solid instead of a liquid culture medium and by selecting for strains that can grow in darkness (*2*). The carrot-cell system is also advantageous because large numbers of cells can develop in a minimum of space and suitable controls can easily be performed.

A carrot-cell culture experiment was placed aboard the unmanned Vostok biosatellite Kosmos 782, launched on 25 November 1975 in the Soviet Union (*3*). The principal objective of this experiment was to test whether such cells could develop normally under conditions of weightlessness in space.

The cells were contained in small plastic petri dishes encased in special canisters (*4*). For 20 days in space one group of cells was exposed to weightlessness, while another group, by means of a centrifuge aboard the spacecraft, was exposed to a gravitational force equivalent to that on the earth. The cells used in this set of experiments were somatic cells of wild carrot (*Daucus carota* var. *carota*, Queen Anne's lace). They had been grown through many cell generations and transfers in flasks which were revolved slowly about a horizontal shaft so that the cells were protected from an asymmetric gravitational stimulus (*5*). They had few visible characteristics of

Table 1. Comparison of degrees of embryonic development achieved by totipotent cells of carrot at 0*g* and 1*g* aboard Kosmos 782. Numbers refer to organized structures subjectively categorized as stages 1 to 4 (*8*). Each value is the average number of individual forms counted per dish in a sample of five dishes ± the standard error.

Experimental treatment	Individual forms per dish				Total forms per dish
	Stage 1	Stage 2	Stage 3	Stage 4	
Flight, 0*g*	1221 ± 336	336 ± 119	152 ± 65	94 ± 37	1803 ± 299
Flight, 1*g**	1131 ± 263	269 ± 45	173 ± 44	98 ± 26	1671 ± 239

*Centrifuge on board spacecraft.

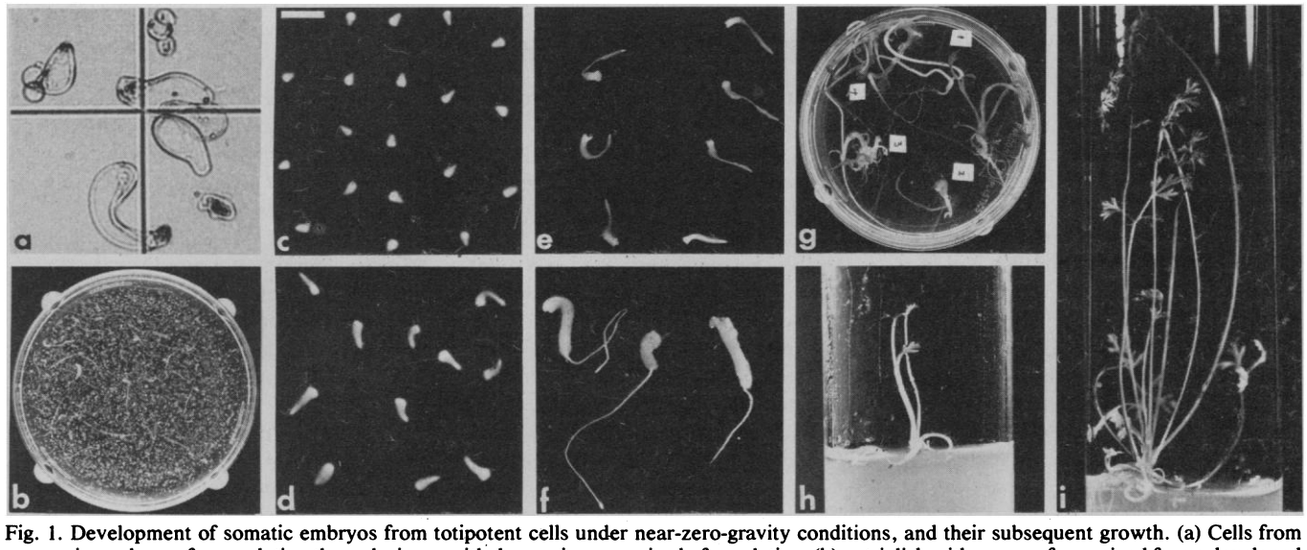

Fig. 1. Development of somatic embryos from totipotent cells under near-zero-gravity conditions, and their subsequent growth. (a) Cells from suspension culture after gradation through sieves with decreasing pore size before plating; (b) petri dish with a crop of organized forms developed after 20 days in space at 0*g* (dish diameter, 50 mm); (c to f) representative embryos removed from dish (scale bar, 0.5 cm); (g) dish with embryos which developed during flight, were transplanted to fresh medium after retrieval, and were grown for approximately 1 month; (h) plantlet as from (g) at time of initial transfer, in culture tube (35 mm in diameter); and (i) plantlet in (h) after 40 days of subsequent growth.

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the differentiated mature somatic cells of their origin and were totipotent. These cultures were filtered through graded sieves that passed only cells or cell clusters on the order of 74 μm in size (Fig. 1a), and these units were distributed uniformly throughout a thin layer of agar culture medium which allowed them to grow heterotrophically in darkness (*6*).

It had earlier been shown that such cultures could withstand the simulated physical hazards of lift-off, flight, and recovery (*7*). A low temperature (4°C) was used to minimize biological activity, especially cell division, before launch and after recovery. It was anticipated that once on the spacecraft, where the flight temperature would be 20° to 24°C, metabolic activity, cell division, and growth would resume and morphogenetic potential could be expressed to whatever extent conditions in flight permitted. As controls, dishes comparable to those in flight were maintained at NASA Ames Research Center, Moffett Field, California; at Stony Brook; and in the Soviet Union.

Viable biological material was recovered from the satellite after flight (Fig. 1b). Such data as photographs of the dishes, numbers of developed structures contained in the dishes, and photographs of embryoids at different stages of their development (Fig. 1, a to f) showed that cells flown at 0*g* produced viable embryos in large numbers and that they did not differ significantly from those in dishes at 1*g*.

The particular culture of carrot cells used contained units which, under the conditions of the experiment, varied greatly in the level of the development they achieved. These were classified by size and complexity into several categories and counted (*8*). Representative samples of the different stages of embryogenesis were photographed and examined microscopically. It was possible to establish statistically that the 0*g* and 1*g* treatments did not result in significantly different proportions of embryos at the different stages of development (Table 1). Cellular units, on the order of 74 μm in size (Fig. 1a) and grown for 20 days in darkness, produced embryos at various levels of development (heart, torpedo, early cotyledonary; see Fig. 1, c to f). Embryos that were more advanced had longer roots and better-developed hypocotyls (Fig. 1, b and f). Shoot development, however, tended to be arrested. Nevertheless, the leaf primordia and shoot apices had been initiated and sufficiently established during the flight, at both 0*g* and 1*g*, so that when embryos from the flight were exposed to 1*g* on the earth, their shoots developed rapidly and normally into plantlets (Fig. 1, g to i). Such plantlets subsequently have been raised to maturity.

It is concluded that totipotent somatic cells can undergo morphogenesis to produce fully competent embryos as effectively at 0*g* as at 1*g*. There are, however, two reservations. First, the imposed conditions (total darkness and the duration of the experiment) do not preclude the possibility that the later rapid differentiation of shoots might be more sensitive to 0*g* than the induction of initial embryonic form. Second, it is still possible, although perhaps unlikely, that the cells used in the experiment had retained some consequence of the asymmetric 1*g* stimulus, so that when they did develop at 0*g* in space, they did so with a "memory" of prior or 1*g* conditions. Moreover, only one generation of cells grown to plantlets was studied, and it therefore remains to be seen whether successive generations could be reared in space (*9*).

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References and Notes

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3. *Aviat. Week Space Technol.* **101** (No. 24), 22 (1974); *ibid.* **103** (No. 12), 20 (1975).
4. Each of two experimental canisters contained a stack of nine 50-mm-diameter plastic petri dishes (Falcon No. 1006).
5. Cells were grown in darkness on apparatus similar to that described by F. C. Steward, S. M. Caplin, and F. K. Millar [*Ann. Bot. (London)* **16**, 57 (1952)] in specially designed culture flasks [F. C. Steward and E. M. Shantz, *The Chemistry and Mode of Action of Plant Growth Substances*, R. L. Wain and F. Wightman, Eds. (Butterworth, London, 1956), p. 167] containing 225 ml of White's basal medium supplemented with coconut water (10 percent) and naphthaleneacetic acid (2 mg/liter), and enzymatically digested casein hydrolyzate (0.025 percent).
6. The plating medium consisted of the basal medium of T. Murashige and F. Skoog [*Physiol. Plant.* **15**, 473 (1962)], supplemented with inositol (20 mg/liter) and sucrose (3 percent).
7. J. Tremor, *1975 Joint U.S./U.S.S.R. Biological Satellite Mission: Project Development and Support* (NASA Ames Research Center, Moffett Field, Calif., 1976).
8. The organized forms observed were separated into four sequential stages of embryonic growth as follows: stage 1, heart-shaped forms; stage 2, torpedo-shaped structures, generally less than 0.75 mm long; stage 3, advanced embryonic forms with a distinct root, generally between 0.75 and 1.5 mm long; and stage 4, small plantlets, each with a well-developed root, generally longer than 1.5 mm.
9. This problem has importance for both space biology and "space agriculture" with flowering plants. It is hoped that this question will be submitted to further tests in space.
10. We could take advantage of the opportunity to use Kosmos 782 only because of long prior experimentation to adapt the experimental system to the anticipated conditions of space flight. This was made possible by a series of NASA contracts initially to one of us (F.C.S.) at Cornell University and later to both of us for our collaboration at Stony Brook. All the work before and after the Kosmos 782 flight involved the indispensable help of F. R. Dutcher and E. T. Yim (now Lau). On all matters concerning engineering design, pretesting of the experimental system, and testing of the package as installed in the spacecraft, we worked in close collaboration with staff members at NASA Ames Research Center, especially J. W. Tremor and R. C. Simmonds. After the Kosmos 782 flight plan was initiated, we enjoyed the full cooperation of the appropriate representatives of the Soviet Union, especially in the Institute of Biomedical Problems and the K. A. Timiriazev Institute of Plant Physiology, directed by O. G. Gazenko and A. L. Kursanov, respectively. R. G. Butenko, of the Timiriazev Institute, made available to us the resources of her laboratory and also functioned as our scientifically informed contact in the Soviet Union.

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Orangutan Death and Scavenging by Pigs

Abstract. *Pongid remains are rarely recovered from tropical rain forests. Observations of a Bornean bearded pig* (Sus barbatus) *scavenging an orangutan* (Pongo pygmaeus) *carcass and the recovery of an orangutan skull fragment corroborates evidence from Africa and suggests that the scavenging of wild pigs may play an important role in the destruction of pongid remains.*

Despite recent taphonomic and primatological field studies, there is still a paucity of data on natural death among the great apes and on the fate of their remains. Such data would help in the interpretation of hominoid remains collected from the paleontological record.

Dying pongids apparently retreat under dense vegetation or stay in nests; for this reason they are usually difficult to locate and observe. Aged or ill individuals frequently disappear without any remains being recovered. Both Schaller (*1*) and Fossey (*2*) found mountain gorilla (*Gorilla gorilla beringei*) skeletal remains in the moist montane forests of the Virunga volcanoes, but it was not clear what processes had modified the carcasses after death. Although chimpanzee (*Pan troglodytes schweinfurthii*) deaths from disease, injury, and related causes have been observed at Gombe National Park, chimpanzee carcasses were rarely recovered (*3, 4*). This was attributed to rapid destruction of the remains by scavengers, insects, and microorganisms. In one exceptional case, a chimpanzee corpse was found within a minute or two

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