In discussing our paper (*1*) Sclar and Carrison (*2*) postulate an interesting alternative mechanism to explain weakening of experimentally deformed minerals and rocks in the presence of water. For various reasons we do not believe that this mechanism operates to any significant extent in our shearing tests.

They contend that shear weakening of the serpentinite and serpentinized dunite occurs *abruptly* above 30 kb in the temperature range 300 to 520°C, where the stability field of serpentine is preempted by a 10 Å layer–silicate phase. Although more pronounced at 30 kb, weakening is not abrupt, but occurs at pressures well below 30 kb. At 15 kb (*1*, Fig. 2) the strength of unserpentinized dunite and synthetic forsterite is greater than the strength of serpentinized dunite. The specimen is more tightly confined at higher pressures, which fact explains the apparent increase in weakening.

We did not specify the dehydration temperature of the serpentine, but, as we stated, those pellets sheared above 450°C were damp after removal from the press. The x-ray patterns also show that the serpentine begins to break down near 500°C. It is notable that Handin (*3*) found weakening in serpentine at temperatures as low as 200°C. Unfortunately, it is not known what effect the composition of the serpentine has on dehydration temperature in our tests; nor do we know which serpentine minerals are present in the samples.

Lastly, the funerary suggestions of Sclar and Carrison were fulfilled: postmortem studies were made of more than 130 sheared pellets shortly after each test was completed; studies included a thorough x-ray examination, using diffractometer and film methods as well as microscopic observations. No brucite, periclase, or 10 Å layer–silicate phase were identified at any pressure, temperature, or shearing condition attained in the tests. Since receipt of Sclar and Carrison's rebuttal (*2*), we have made other, longer-time tests (45 minutes) on serpentinite in the region above 30 kb at 450°C. The x-ray patterns did not show a 10-Å phase. Since most of our shearing tests were accomplished rapidly, usually within 10 minutes, we think it unlikely that sufficient time would be available for nucleation and growth of a new phase. It may be important in this respect that Sclar and Carrison used reactive oxide mixtures in their experiments, while our starting materials were natural or synthetic minerals.

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Single Cells, Coconut Milk, and Embryogenesis in vitro

Steward and his co-workers (*1*) reported obtaining thousands of embryos from carrot cell suspensions grown in media containing coconut milk. This fact was taken as evidence for his hypothesis (*2*) that isolated cells tended to behave as if they were zygotes when exposed to media containing coconut milk (the liquid endosperm that normally nourishes the coconut embryo). The belief that coconut milk has embryogenic effects on single plant cells in culture has now achieved the status of accepted truth. Perhaps the final approbation has been given this theory in *Plant Biochemistry* by Bonner and Varner (*3*). Bonner states, "Two conditions must be satisfied for this to occur [embryogenesis]. The specialized cell must be separated from its neighbors, that is, it must be a single cell. In addition, the cell must be surrounded by medium which contains the nutrients needed for embryo growth. The liquid endosperm of coconut or horse chestnut contains the required substances. If either of the two conditions is not satisfied, embryos are not produced. Thus, if clumps of cells, rather than single isolated cells, are placed in the enriched medium, they grow into undifferentiated masses of callus. If the embryonic nutrients [coconut milk, and such] are omitted, no growth takes place."

The categorical statements made by Bonner are surprising, to say the least, since Steward's theory has never been supported by experiments showing that coconut milk has the purported effect, or that the embryos in cell cultures must be derived from single cells. In fact, it has been shown conclusively that neither coconut milk nor any other similar nutrient complex is required for embryogenesis in carrot cell cultures (*4, 5*), and the available evidence indicates that embryos in cultures of carrot and other species usually develop from cell clumps—not from single cells (*6, 7*).

I offer the following points to support this contention:

1) Although Steward has obtained embryos from cell suspensions of the wild carrot, *Daucus carota*, grown on media containing coconut milk (*1*), he has not reported control experiments establishing that coconut milk is the component of the medium responsible for embryogenesis. Steward's data which showed an absence of cell division in the absence of coconut milk (*1*) hardly comprised an adequate control, since the basal medium used was designed 30 years earlier for growing tomato root cultures (*8*) and has too little of nitrogen and several other minerals for adequate growth. Experiments of others show that embryogenesis occurs readily in wild carrot cultures started and maintained through numerous transfers on media containing only minerals, sucrose, vitamins, and an auxin (*4, 5, 7*). If there are special "embryonic nutrients" involved, they must be produced by the cells themselves, a fact that has theoretical implications different from those of Steward's theory.

2) In cultures of other varieties of carrot (*9*) and in other species (*10*) where embryos have occasionally been observed, either coconut milk was absent from the medium or the essential components of the medium were not identified by control experiments. In short, although coconut milk has an unusual capacity for inducing growth in explants and is often used in culture media, it has never been shown to have any relevance for embryogenesis in vitro. On the contrary, in the only well-studied experimental system, the wild carrot, coconut milk has been shown to inhibit embryogenesis partially or completely, depending on the cultural circumstances (*4, 5, 7*). Abnormal development of young embryos in the presence of autoclaved coconut milk was demonstrated by Van Overbeek in 1942 (*11*).

3) Steward has not provided data to support this contention that single cells were the source of embryos in his cultures. The available evidence indicates that mitosis in plant cell suspensions

occurs largely in the cell clumps always present (*12*) and that the single cells found in such cultures arise by sloughing off the clumps. Nearly all of these single cells die and become part of the debris in the culture vessel. Figure 1 shows such clumps from carrot cultures and the manner in which one or more embryos arise from them. These clumps are self-perpetuating through continued growth and fragmentation. If cultures are started from a sieved inoculum which contains over 95 percent single cells and a small percentage of clumps 2 to 5 cells in size, the number of embryos obtained is about equal to the number of clumps originally present in the inoculum (*7*). Studies of ultrastructure reveal that even the smallest clumps in the inoculum, which appear in the light microscope to have been derived from single cells, show wall fragments where they have torn off a parent clump (*13*).

I do not dispute that single cells can and do give rise to embryos under certain conditions where they are capable of mitosis. A small percentage of single cells may divide in newly established suspension cultures during the first passage on liquid media. Within the intensely conditioned interior of a callus mass, single cells undoubtedly divide and give rise to embryos. We have also observed that embryos occasionally form in microcultures containing groups of single cells on conditioned media (*5*). The critical point is that the presence of viable, isolated cells in liquid cultures, rather than being a "requirement" for embryogenesis, is a rare event.

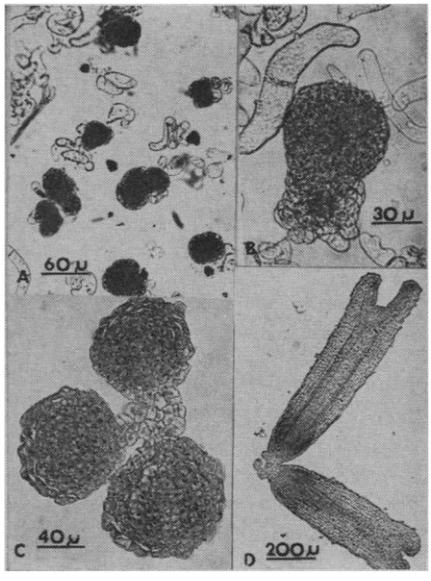

Fig. 1. *A*, Clumps in the 45- to 75-μ size range sieved from carrot suspension cultures. Clumps washed free of auxin develop either a single embryo (*B*) or multiple embryos (*C*). *D*, Mature embryos still attached to the original clump.

4) Of equal significance are the numerous examples known of the origin of embryos from one or more cells of the ovule which are not isolated but which lie within well-organized tissues such as the nucellus or integuments (*14*). A similar case is seen in the report, well-supported by histological data, of the development of embryos from epidermal cells of *Ranunculus* (*15*). Although such cells may be "physiologically isolated" and thus not subject to the normal controls exerted over cells in tissues, the concept of physiological isolation is not definable in terms of cell chemistry and contributes little to our understanding of the phenomena described. Furthermore, since alternative developmental pathways are open to cells which become isolated from normal controls, such as their differentiation in vitro into tracheids or the production of buds and roots (*16*), isolation per se should not receive undue emphasis as a factor in embryogenesis.

5) A final point concerns the implications of Steward's theory for embryo development from the fertilized egg. The theory clearly implies that in the angiosperm embryo sac the zygote is a mere pawn, which is induced to begin its development by the presence of endosperm, and is directed into its particular developmental pathway by endosperm. In other words, a minimum of morphogenetic control is assumed to reside within the zygote. Many embryos do digest away the endosperm as they grow. In such cases, if the endosperm fails to form, the embryo usually aborts. Does this mean that the endosperm exerts a "morphogenetic" control over the zygote and causes it to develop into an embryo rather than into something else? If the sucrose or minerals are omitted from wild carrot cell cultures, embryos also fail to form, clearly as a result of nutritional and not morphogenetic failure. The same interpretation might be applied to the abortion of embryos in ovules lacking endosperm. It is significant that there is no endosperm in several angiosperm families (*14*), and in many apomictic species adventive embryos begin their growth before the endosperm exists (*17*).

Thus the stimuli enabling cells to serve as embryo initials, whether in vivo or in vitro, act in advance of the formation of "embryonic nutrients." Furthermore, while isolation from normal controls may be a requirement for the expression of embryological competence, it does not appear to be the cause of such competence. The nature of the stimuli involved and of embryological competence itself remains obscure. In future experiments designed to study the control of embryo induction in cell cultures, we should distinguish between what has been established and what must remain as hypothesis based on inadequate data.

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