of a contaminant of the cesium chloride. The contaminant persisted after treatment of the solution with activated charcoal and recrystallization of the salt. It was relatively insoluble and showed yellow fluorescence when examined under ultraviolet light.

Our results establish that there are two categories of DNA in *Leishmania* and indicate that the small quantity of the less-dense band of DNA is located in the kinetoplast. It is not possible to state categorically that no minor component exists in the nucleus. However, it would appear that little or no major component is present in the kinetoplast. The rapid equilibration of the minor component suggests an unusual configuration or aggregation such that it responds to the centrifugal force more rapidly than the DNA of the nucleus and *E. coli*. In view of this behavior, the guanine-cytosine content of the minor band might not be 36 percent as calculated from the apparent density, 1.699, by the equation of Sueoka *et al.* (*5*).

To date, three pieces of evidence support the contention that the minor DNA component is of lower molecular weight than the major DNA component and that its rapid banding results from its having a more favorable shape factor than the major component. (i) When both types of DNA were sheared by sonication (*6*), as evidenced by extremely slow formation of broad bands, both broad bands were found at the same density as their intact counterparts. (ii) After being equilibrated in cesium chloride the rotor was stopped for 3 hours and then started again; despite the rotation of the cell contents from a vertical to a horizontal position upon stopping the rotor, when the rotor again reached 44,770 rev/min the major band was observed in its equilibrium position, although now about twice its equilibrium breadth. The minor band had vanished, presumably because of diffusion or mixing, or both, but it re-formed within an hour in its usual fashion. (iii) When whole *Leishmania* DNA dissolved in a solution containing 0.015*M* NaCl and 0.0015*M* sodium citrate was subjected to velocity sedimentation, the major absorbing band sedimented more rapidly than a minor component.

It is of interest that the chloroplast-containing algae *Chlamydomonas reinhardi*, *Chlorella ellipsoidea*, and *Euglena gracilis* have major and minor bands of DNA, the concentration and density of which are very similar to the DNA of *Leishmania* (*7*). The trypanosomatid flagellate *Crithidia oncopelti* (*8*) contains a satellite band of the same density as the *Leishmania* minor DNA band. It would be of interest to know if these various minor bands of DNA, and also the DNA of the crab testis (*5*), form bands as rapidly in cesium chloride as does the DNA from *Leishmania* kinetoplasts.

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4 January 1965

Ontogeny of Adventive Embryos of Wild Carrot

Abstract. *Somatic carrot cells in culture divide to produce undifferentiated preglobular proembryos which exhibit a wide variety of segmentation patterns. One or more globular proembryos, which exhibit normal histological zonation, may develop from single preglobular proembryos. Regeneration of normal embryos from cultured cells grown on media containing only minerals, sucrose, vitamins, and 2,4-dichlorophenoxyacetic acid suggests that the embryo sac may have less of a formative role than currently ascribed to it.*

The recent demonstration that cultured somatic cells of the wild carrot *Daucus carota*, when grown on simple defined media, will regenerate carrot embryos focuses attention on the forces which direct embryo development (*1*, *2*). The view that embryo development is under considerable regulation by unique physical and chemical factors in the embryo sac (*3*) gained support from experimental studies which purported to show that coconut milk induced cultured cells to behave as zygotes (*4*). However, the occurrence of embryogenesis in the presence of coconut milk does not establish a causal relationship.

It is now clear that coconut milk is not required for the regeneration of embryos from cultured cells of the wild carrot and is, in fact, inhibitory to the development of carrot proembryos (*2*). It is also significant that in the only well-documented report of embryos from cultured cells of the domestic carrot (*5*), coconut milk was not used in the medium. This point needs to be emphasized since studies of the molecular basis for the regeneration of embryos from cultured cells will be hampered by the use of such complex substances as liquid endosperm.

We now present evidence that embryos which develop from wild carrot cells grown on a basal medium (*2*) containing only minerals, sucrose, vitamins, and 2,4-dichlorophenoxyacetic acid (2,4-D) undergo a series of developmental changes which may be more similar to embryogenesis in the ovule than previously reported, and that variations in the ontogeny of undifferentiated proembryos, caused by culture conditions, have little effect on subsequent embryo development.

We stated previously (*1*) that adventive embryos differed from seed embryos in that they usually did not have a suspensor-like component. This conclusion was erroneous, and attributable to the fact that most observations were made of embryos dissected from callus or embryos which developed in rotating liquid cultures—circumstances which led to the loss of the fragile suspensor (*6*). We have now studied numerous microtome sections of embryos embedded in callus in the original position where they formed, and it is evident that nearly always a distinct suspensor of variable size and shape is present (Fig. 1). In addition, we have grown groups of single cells and observed the entire developmental sequence from single cells to mature embryos. Evidently, the suspensor is nothing more than those cells of the proembryo (*7*) which are not incorporated into the embryo proper, and the size and shape of the preglobular proembryo varies, depending upon the number and orientation of mitoses which occur in random fashion prior to the establish-

ment of polarity. Under the culture conditions used, polarized growth and histological differentiation were inhibited if proembryos were exposed to a concentration of 2,4-D higher than 0.1 mg/liter. When polarity was established in a preglobular proembryo of few cells, subsequent growth and differentiation usually resulted in a globular proembryo with an insignificant suspensor (Fig. 1*C*). If polarity was delayed until a sizable cell mass developed, a single globular proembryo with a massive suspensor (Fig. 1, *A* and *B*) might differentiate, or, commonly, two or more embryos might develop from the same cell mass. In the latter case, the multiple embryos shared a common suspensor and were thus joined at the radicle (Fig. 2).

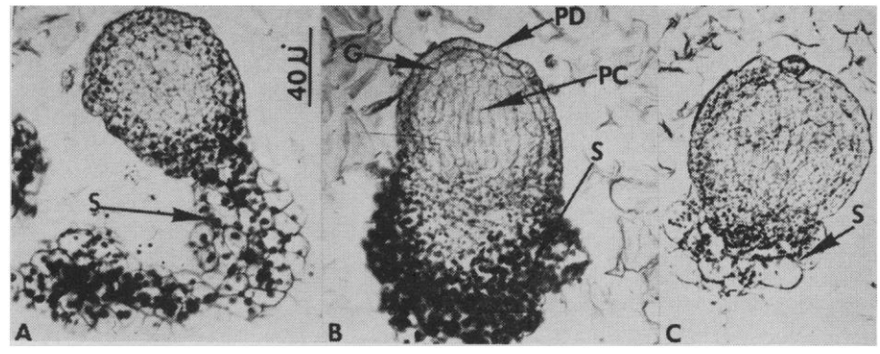

Fig. 1. Sections (15 μ) of globular proembryos embedded in callus. *A*, A proembryo which has differentiated at one end of a massive filament which contains starch grains; *B*, a proembryo which has developed from an undifferentiated spherical mass of cells which are dense with starch; *C*, a proembryo with ephemeral suspensor and relatively little starch (*S*, suspensor; G, ground meristem; *PD*, protoderm; *PC*, procambium; scale in *A* applies to all three embryos).

These variations in ontogeny are illustrated in Fig. 2. Small starch-filled cells divide to form preglobular proembryos which may be spherical, filamentous, or irregular in shape—the particular segmentation pattern being a response to random chemical and physical factors in the callus mass or cell suspension where such proembryos develop. The disorganized spheres (Fig. 2*b*) generally develop into single embryos, as shown by the sequence *b*, *e*, *h*, in Fig. 2. Although the well-organized filamentous proembryos appear to have axial polarity and may proliferate at one pole to yield a single embryo with a filamentous suspensor (Fig. 1*A*), unrestricted access to nutrients can also lead to proliferation at several points on the filament rather than just at one end. This type of development is illustrated by the dumbbell-shaped structure in Fig. 2*f*, which consists of two globular proembryos that have developed at either end of a massive filament. Proliferation at the center of such a structure would lead to the triplet shown in Fig. 2*g*. Mature embryos united at their (radicle) ends, as shown in Fig. 2, *i* and *j*, are commonly found in tissue cultures. Cleavage polyembryony, in which multiple embryos develop from an irregularly shaped or branched filamentous proembryo, has been reported as a common occurrence in the embryo sac of several Angiosperm genera (*8*).

Regardless of the segmentation pattern which results from random growth of the preglobular proembryo, the globular proembryo which ultimately differentiates shows normal histological organization. Figure 1 illustrates the normal tissue zonation of three globular proembryos which have obviously had different ontogenetic histories. Protoderm, ground meristem, and procambium are readily discernible. The embryos in Fig. 1, *A* and *B*, show prominent starch grains in the suspensor; the embryo in *A* also shows starch in the region of the ground meristem; that in *C* shows little starch. Starch grains are usually found in the cells of the preglobular proembryo and disappear as the globular embryo differentiates, disappearing first in the region of the procambium. Often, however, numerous grains are present in the cortical region of mature embryos.

Single cells and proembryos containing fewer than five cells were sieved from freshly isolated callus (one to three subcultures) in large numbers, thoroughly washed, and plated on fresh basal media according to the techniques previously described (*2*). Embryogenesis occurred readily under these circumstances. Similar culturing of cells derived from callus which had undergone prolonged subculturing did not yield embryos. Attempts to stimulate embryo-

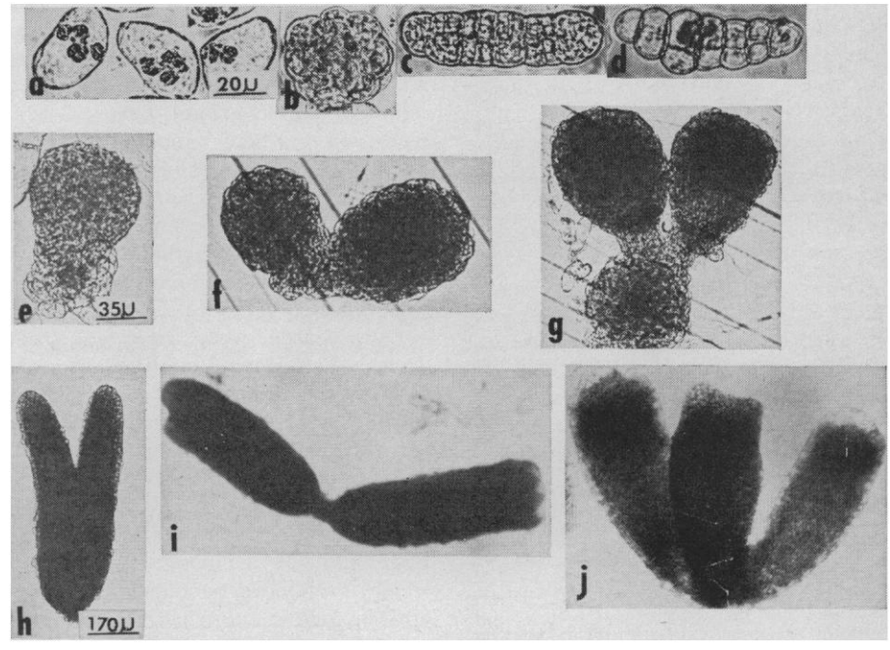

Fig. 2. Various stages in the ontogeny of adventive carrot embryos. Starch-filled embryo initial (*a*) develops into preglobular proembryos which are either spherical (*b*) or filamentous (*c* and *d*); the filamentous pattern is typical of proembryos in the carrot family. Spherical preglobular proembryos (*b*) usually develop into single embryos (*e* and *h*), whereas filamentous proembryos may proliferate to yield either one embryo as shown in (*e*) and (*h*), or multiple embryos as shown in (*f*), (*g*), (*i*), and (*j*). Multiple embryos are commonly found in tissue cultures along with single embryos. The scale, 20 μ in (*a*) applies to (*b*), (*c*), and (*d*); 35 μ in (*e*) applies also to (*f*) and (*g*); 170 μ in (*h*) applies also to (*i*) and (*j*).

genesis in the uniform parenchymatous cells of these older cultures, by adding coconut milk (10 percent by volume), carrot root extracts, or mixtures of natural and synthetic growth regulators to the medium, occasionally resulted in the development of embryos. However, these few embryos are now believed to have been the result of encouraging the growth of a few proembryos carried over from earlier subcultures, rather than the result of inducing some change in "noncompetent" cells.

These findings indicate that cells which are embryologically competent can serve as the starting point in a developmental pathway in which the morphogenetic controls are largely internal. In other words, developing carrot embryos do not appear to require a complex array of exogeneous nutrients or an environment with precise chemical and physical gradients. The factors leading to the "competency" of the zygote or of certain cultured cells are unknown (*9*).

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7. The term suspensor here refers to those cells of the proembryo which are not incorporated into the embryo proper, and which thus have the same topological significance as the suspensor cells of seed embryos. Virtually nothing is known of the function of the suspensor, other than its possible role as a device for pushing the embryo into the endosperm, so no real homology can be established.
8. We have adopted a modification of the terminology of Soueges, in which the term proembryo refers to any developmental stage preceding cotyledon initiation [R. Soueges, *La Differenciation* III. *La Differenciation Organique* (Hermann, Paris, 1936)]. The term preglobular proembryo refers to the histologically undifferentiated condition preceding the initiation of protoderm, and globular proembryo refers to the stage following protoderm initiation and preceding cotyledon development.
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10. Since this paper was submitted, we have found that for embryogenesis to proceed in vitro the presence of ammonium ions is essential. Callus will grow indefinitely on a medium containing nitrate alone, but segmentation patterns typical of proembryos will not form. Preliminary experiments indicate that glutamine, aspartate, a mixture of amino acids, or coconut milk will not substitute for the ammonium requirement.
11. This work was done during the tenure of an NIH fellowship (W.H.) and with the aid of NSF grant GB-1936 to D.F.W.

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Brightness Discrimination in the Collared Lizard

Abstract. *Eight collared lizards were tested on a modified Lashley apparatus for brightness discrimination. The animals reached the criterion level of learning in 335 trials and exhibited behavior on the choice platform comparable to that of the rat presented with a similar problem. The pattern of latency appears to be atypical in the lizard, since there was little decrement throughout the learning period.*

Snakes and turtles have served as subjects in most studies of the problem-solving abilities of reptiles. Although various aspects of behavior have been studied in lizards, very little is known about their ability to modify stereotyped behavior by learning. Here we report the behavior of eight collared lizards (*Crotaphytus collaris*) when presented with a brightness-discrimination problem.

The collared lizard occurs in rocky desert areas over much of the western United States, and its ecology is well known (*1*). It is particularly suitable for experimentation; it is large enough to be handled with ease, and its pugnacious temperament at the time of capture is readily transformed into docility after brief periods of handling. Most important, it shares with other heliothermic reptiles the tendency to maintain, by behavioral means, a relatively constant body temperature during periods of activity. Thus the question of motivation and reward, so often a problem with ectothermic animals, is resolved by the use of heat, for which the animals show no apparent satiation with repeated exposure.

Our lizards were collected from Townes Pass, Inyo County, California, at elevations between 700 and 1100 m. The mean body temperature of the experimental animals in their natural habitat was 37.1° ± 1.8°C, which approximates the optimum temperature for collared lizards as indicated by Fitch (*1*). A temperature of 40°C, which is often encountered by the lizards in their natural habitat, was provided as a reward in the experiments. Exposure to this temperature for a period of 3 minutes causes a significant rise in body temperature. The five male and three female lizards used in the study varied in snout-vent length from 70 to 90 mm with an average length of 80 mm. The animals were sexually mature, and all either maintained or gained weight while in captivity.

The lizards were housed in screened wooden cages with sand and rocks on the floor. They were exposed to heat for 3 hours in both the morning and evening. The heat was provided by 250-watt infrared lamps suspended 105 cm above the cage floor. Fluorescent lamps were usually on during the day and the room was dark at night, but there was no attempt to regulate the photoperiod. Food, consisting entirely of meal worms (*Tenebrio* larvae) during the period of training, was given only when the lizards were in the experimental apparatus. Water was provided in the home cages and was available in the small glass tanks used to hold the animals during the trial periods.

The experimental apparatus was a modified Lashley discrimination stand (Fig. 1), 95 mm high, covered with clear plastic 3 mm thick to prevent escape of the animals. A hardware cloth ramp led up to a "choice platform" from which the animal could view the two goal boxes; the floor of the platform was covered with neutral-colored sandpaper which provided a tractional surface. Alleys separated by a 30-deg angle extended to the goal boxes. One alley and the positive goal box to which it led were lined on all surfaces with sandpaper painted with white enamel; the negative goal box and its alley were similarly lined in black. Attached to the floor of both alleys were two electrode plates extending from wall to wall. The electrodes across the white alley were inactive. Those across the black alley were powered with a

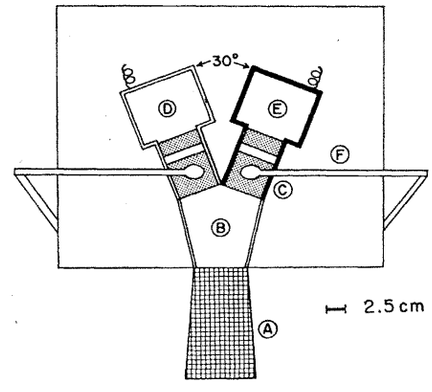

Fig. 1. Diagram of discrimination apparatus. *A*, Screen ladder; *B*, choice platform; *C*, alley with plate electrodes; *D*, positive goal box; *E*, negative goal box; *F*, lamps extended over alleys.