Morphogenetic Interaction of Presumptive Neural and Mesodermal Cells Mixed in Different Ratios

Abstract. *Cells of the presumptive forebrain region and axial mesoderm of* Triturus *neurulae were disaggregated and combined in different ratios. The differentiation of the central nervous system in these explants was dependent on the relative amount of mesodermal cells present: an increase of mesodermal cells resulted in a corresponding increase in the frequency with which caudal structures of the central nervous system developed and a gradual loss of the forebrain formations.*

In 1955 we formulated a hypothesis of the inductive mechanism underlying the sequential determination of the central nervous system (*1*). The original concept was based on experiments in which two heterogenous inductor tissues, one leading to forebrain determination and the other to formation of presumptive mesoderm from gastrula ectoderm, were used simultaneously as implants. As a consequence, a combined action was observed, and caudal structures of the central nervous system (CNS) were induced; these structures could not be detected when either of the two were implanted separately. Subsequent experiments in which two such different inductors were combined in different ratios supported the hypothesis (*2*); a gradual increase of the tissue that induces formation of mesoderm resulted in a caudal shift of the neural structures induced by the tissue that brings about formation of neural components. It was therefore suggested that, when acting alone, the factor or factors that induce neuralization would lead to the induction of forebrain structures only, whereas interplay with the principle that induces formation of mesoderm would result in the formation of hindbrain and spinal cord (*3*). The sequential nature of this determinative process was subsequently demonstrated in the following experiments (*4*). Since it is known that primary inductors localized in the invaginating blastoporal lip can act upon the ectoderm only for a limited period of time [less than 24 hours (*5*)], predetermined cells (artificially induced to neural or mesodermal direction) were disaggregated after this period of competence and cultivated either as separate aggregates or as mixed populations. The results closely resembled those obtained with combined heterogenous inductors and thus strongly suggested that the final regional specialization of the CNS was controlled by interactions of the two types of predetermined cells. That is to say, the neuralization determined during the initial steps of induction had no stable regional character and could be altered by subsequent morphogenetic stimuli. Both artificially induced mesoderm and cells of the mesodermal layer of the embryo possessed this capacity to control the specialization of the CNS (*6*). Consequently, the results obtained with two inductors mixed in different ratios may find its explanation in the different amounts of neuroepithelium and presumptive mesoderm induced by different combinations of the two inductors. This suggestion has now been tested experimentally.

The neuroepithelium of the forebrain region, with some adjacent presumptive epidermis, and a transverse zone of trunk mesoderm were removed from early *Triturus vulgaris* neurulae (stage 13 Harrison) (Fig. 1). The operation was facilitated by first immersing the neurulae in double-strength Holtfreter saline for a short time (*7*). These fragments were combined in different ratios of neuroepithelium to mesoderm: 10 : 1, 5 : 1, 5 : 2, 5 : 5, 2 : 5, and 1 : 5; the combined tissues were then disaggregated together by short-term treatment in ethylenediaminetetraacetate in medium free of Ca and Mg (*8*). The disaggregated cells were thoroughly mixed by repeated pipetting and transferred to small hollows in agar layers on the bottom of culture dishes containing

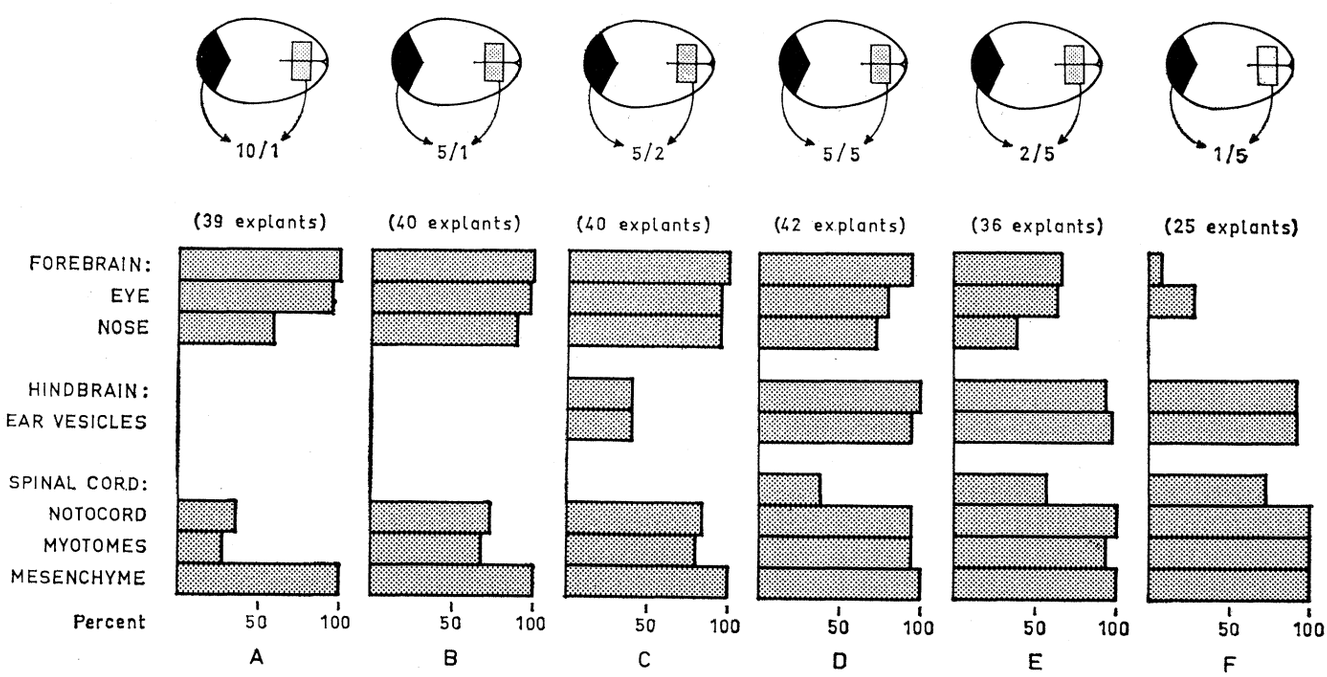

Fig. 1. Distribution (percentages) of different structures in reaggregates cultivated for 14 days in vitro. The scheme indicates the primary isolation of the fragments and the ratios of these in different series.

normal culture medium [Holtfreter-Ringer solution buffered with tris-(hydroxymethyl)aminomethane with 0.1 percent Elkosin (Ciba)]. The reaggregated cell clusters were cultivated in this medium for 14 days at 18°C, fixed, and analyzed microscopically in serial sections.

The results of the analysis of the differentiations in the different series are seen in Fig. 1. In the series with definite neuroepithelial predominance (ratios of neural to mesodermal fragments, 10:1 and 5:1), the brain vesicles could invariably be classified as forebrain structures, frequently accompanied by eye rudiments with or without lenses, isolated lens vesicles, and nasal pits. Intermingled with these neural and epidermal structures were scanty fragments of mesodermal differentiations, representing myotomes or muscle fibers, notochord, and mesenchyme. No structures similar to hindbrain or spinal cord were noted in these two series.

When the proportion of mesodermal fragments was increased (5 : 2), the first caudal structures of the CNS could be seen. Small fragments of hindbrain and ear vesicles were discerned in about 40 percent of the explants, together with well-differentiated and more abundant mesodermal derivatives. In the series in which an equal number of neuroepithelial and mesodermal fragments were combined, hindbrain vesicles were seen in all explants; fragments of spinal cord were found in 38 percent. When the proportion of mesodermal fragments was increased still further (2:5 and 1:5), the frequency of forebrain fragments began to decrease, and in the last-mentioned series only 8 percent of the explants had brain vesicles that could be classified as forebrain. In contrast, hindbrain vesicles were still almost invariably found in both these series, and the frequency of spinal cord increased.

These results seem to corroborate our previous hypothesis on the sequential process leading to the segregation of the CNS. During the initial stage of induction the cells are determined to become neural, but they acquire no stable regional character. This is subsequently controlled by the mesodermal cells and apparently in a quantitative way, since an increasing amount of mesoderm surrounding the neural cells shifts segregation in the caudal direction.

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Phytostat for Continuous Culture and Automatic Sampling of Plant-Cell Suspensions

Abstract. *An apparatus for growing plant cells in suspension culture is described; it may be used for continuous or batch culture, and is equipped with a valve for automatic collection of samples. Aeration is by continuous bubbling of air into the culture through fritted glass. Normal culture-duplication times are from 30 to 35 hours.*

Various systems have been designed to grow plant cells in liquid suspension culture on a larger scale than is possible in shake flasks (*1, 2*); they depend on airflow or container rotation for agitation of the cultures, and have no provision for automatic sampling and feeding of the cells (*1, 2*). Attempts to culture plant cells in the cyclone-column unit (*3*) were unsuccessful because the cells were broken during circulation of the culture.

We now describe a phytostat in which the culture vessel was a 5-liter round-bottomed pyrex flask, modified as necessary (Fig. 1). Agitation was achieved with a Teflon-coated stirring bar held in a Teflon rod supported on a small indentation in the bottom of the flask. The stirrer was stabilized by a stainless steel rod that rotated in a Teflon bushing mounted in the rubber stopper. The stirrer was driven by a magnetic bar mounted on the end of a rod held in a chuck and driven by a variable-speed motor (1/18 hp, 0 to 4000 rev/min, 10 : 1 gear reduction); the speed was varied by means of a Variac. This system eliminated heating of the culture by the motor and provided a constant stirring rate.

The rubber stopper (No. 14) allowed ample room for inlet and outlet probes. The air entered through a tube having a fritted-glass tip; the tube could be adjusted to bubble air through the culture or to aerate the upper portion of the vessel. The airflow was controlled by a pressure regulator and a secondary rate valve, and was measured by a rotameter (*4*). The air was passed through a copper sulfate–water tower to increase the humidity so that less water was lost from the culture; it was sterilized by passage through a 15-cm filter packed with fiberglass.

The position and design of the sampling valve (Fig. 2) provided zero dead volume of culture so that one could select reproducibly small representative samples. A solenoid valve (*5*) was modified to operate the Teflon plunger which was seated in a washer that was clean-cut from a 3-mm sheet of silicone rubber. The orifice of the valve was 3.51 mm in diameter—large enough to prevent clogging and to facilitate uniform sampling.

When the sampling valve closed, a rinse of sterilized water removed the sample from the valve and tube leading to the fraction collector; the rinse entered the valve through a 6-mm stainless steel tube inserted through the end of the solenoid housing (a three-way valve also could be used). The rinse flow was controlled by a second solenoid.

The tube leading to the fraction collector was left open to the atmosphere and remained filled with rinse water. The culture flask was rarely contaminated through this tube, especially if the tube was pinched off when sampling was not automatic.

The sampling program was controlled by two timers in series (Fig. 3). Sampling was frequently governed by an adjustable recycling timer (*No. 1*) (*6*); at the end of its cycle a signal was sent to a latching relay (*7*) that turned on the second timer (*No. 2*)