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Culturing spinal cord explants in a collagen gel

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A new method for culturing spinal cord slices or explants is presented which entails the use of a commercially available purified collagen, Vitrogen. Vitrogen provides a stable three-dimensional matrix for culturing spinal cord explants which is superior to the conventional method of applying explants to moist dishes coated with rat tail collagen. The use of Vitrogen facilitated the culturing of spinal cord explants which remain viable for over 2 1/2 weeks in culture, in addition to enhancing neuritic growth.

Collagen commonly is used as a substrate for culturing cells (Ehrmann and Gey, 1956; Bornstein, 1958; Hauschka and Konigsberg, 1966, Chamley et al., 1973, Kleinschmidt and Bunge, 1980; Dribin and Barrett, 1982). However, it is difficult to insure that most slices or explants of tissue will adhere to the dish. An alternate method for culturing explants is presented which involves the use of a collagen matrix, Vitrogen. Vitrogen is liquid at cool temperatures (5 °C) but rapidly denatures to a gel at warm (37 °C) temperatures. Explants can be suspended in liquid Vitrogen (Vit.) and immediately after plating, the Vit. denatures to a gel which adheres itself and explants suspended in it to the dish. In these experiments fetal rat spinal cord explants were plated either on collagen coated dishes or in Vit. and compared with regard to the number of applied explants that attached to the dishes and the extent of neuritic growth from those explants.

Spinal cords from 13–14-day fetal rats were used. For each hemicord, after removal of the meninges and dorsal root ganglia the dorsal half of the cord was removed and the remaining ventral portion was thinly sliced (approx. 0.5 mm). Excess phosphate buffered saline (PBS) was removed and the explants plated either on collagen coated dishes or in Vit.

Collagen coated dishes were preincubated for 1 h with medium which was removed before the addition of the cord slices. The moist dishes with explants were

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placed in a humidity chamber in a 5% CO₂ incubator (37°C) for several hours to overnight to permit the explants to adhere to the collagen. 1 ml of neuron medium (NM) was gently added, attempting to minimize mechanical jarring of the explants.

Vit. solution was prepared according to Bischoff (pers. commun.; see below) and added to a tube with the drained explants; note that excess dilution affects the gelation. 1–2 drops/dish were added to precooled dishes. The mixture was spread evenly around the plate to the edge. The plates were placed in the incubator (37°C) for 15 min to allow the Vit. to gel, after which 1 ml of NM was added. The Vit. layer was approximately 200 µm thick.

Dissections were done in PBS with 10% glucose, 0.72 mM MgCl₂ and 1.6 mM CaCl₂, pH 7.4. Cultures were fed every other day with NM based on Bottenstein and Sato's (1979) N2 but with 1 mM putrescine, no selenium, 20 mM additional KCl, 10% heat-inactivated horse serum, 5% chick embryo extract, 50 units/ml penicillin, 50 µg/ml streptomycin, 50 µg/ml gentamicin and 0.125 µg/ml fungizone. The collagen solution was prepared sterilely from rat tails according to Ehrmann and Gey (1956) in 1 : 1000 acetic acid solution. Vit. solution was prepared immediately before use by combining in a sterile tube (on ice) 2 ml Vitrogen 100 (Flow Lab.), 0.25 ml 5X Modified Eagles Medium (MEM), 0.10 ml 0.14 M NaOH and 0.08 ml 7.5% NaHCO₃ pH 7.4 (Bischoff, pers. commun.). Vit. solution was kept on ice until added to the spinal cord slices.

Twenty-four hours after plating the dishes were assessed for the number of explants that adhered vs that initially applied. If explants were not secured to the dish, they did not exhibit neurite growth and were removed with the next change of medium. The difference between culture conditions was dramatic. The percentage of explants secured to the dishes was much greater in Vit. than with collagen, 91.1 ± 7.3% vs 13.7 ± 12.1% respectively (n = 38 plates; $\bar{x} \pm S.D.$ total number of explants applied: 796 on Vit. and 435 on collagen). Thus, securing explants to the culture dishes was facilitated with the use of Vit.

The explants in Vit. (Fig. 1) put out more and longer neurites than did the explants on the collagen dishes. Neurite growth was assessed by measuring the length (from the edge of the explant) of representative neurites at 90° intervals around the explant. Thus, the lengths in Table I give an indication of the size of the 'halo' of neuritic growth and not the lengths of the longest neurites. Data are given in Table I for 4 representative plates, 2 each of Vit. and collagen from the the same culturing batch at 7 days after plating. The zero value represents a side of the explant that did not produce neurites. This was observed in roughly half of the explants regardless of culture conditions. Note the several-fold difference in neurite length of the Vit. explants over the collagen ones. This difference was observed in all of the Vit./collagen cultures. Cord explants in Vit. remained viable for over 2 weeks in culture, as determined by subsequent coculturing with muscle for several weeks (see below).

In the present study a higher percentage of explants were successfully cultured with the Vit. gel than with the collagen. Neurite growth was also enhanced in the Vit. cultures. This may reflect inherent differences in the culture conditions, for example, the relatively dry period in the humidity chamber for the collagen plates may result

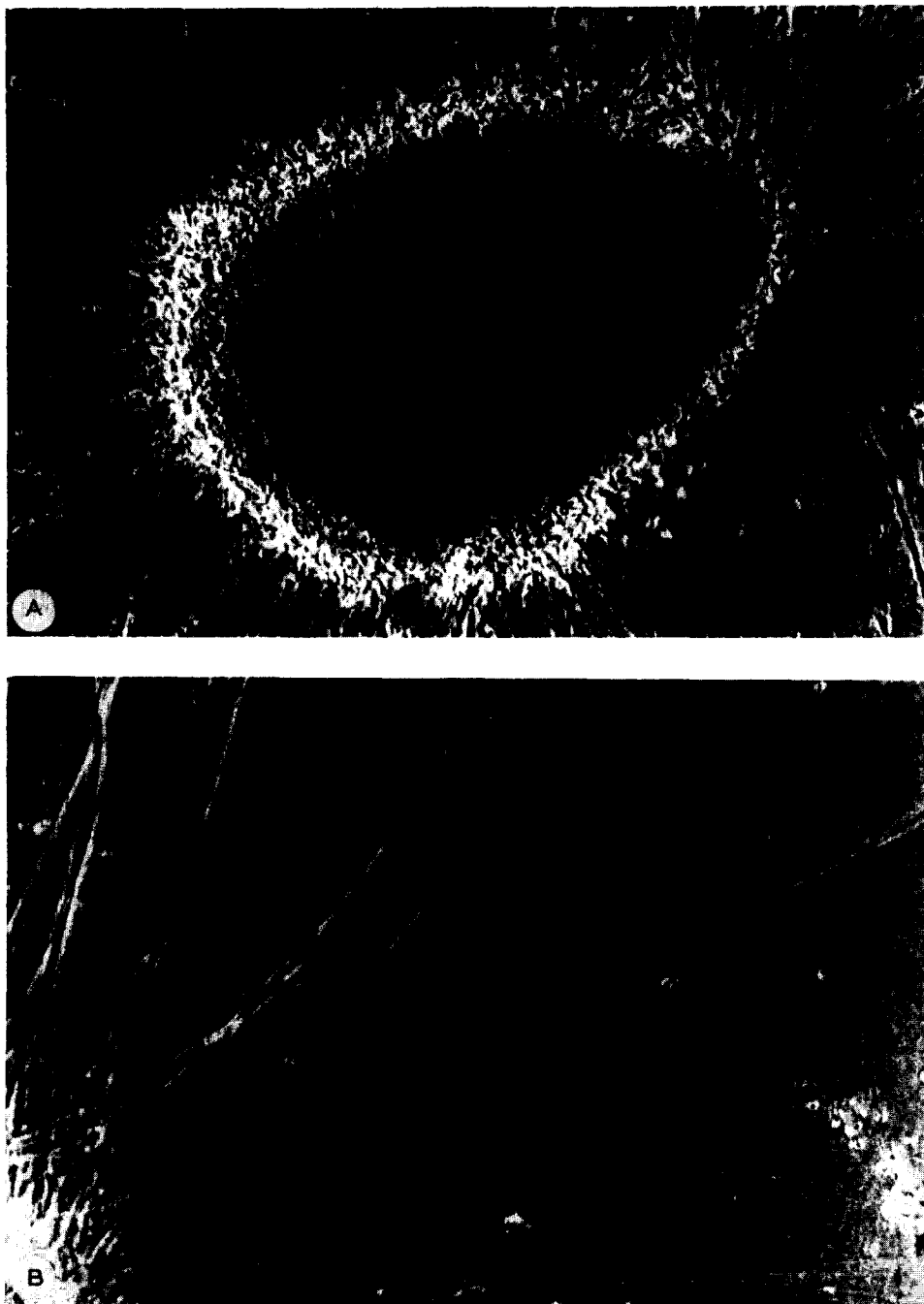


Fig. 1. Spinal cord explant in Vitrogen culture for 3 days. A: phase contrast photomicrograph of explant. Note the extensive neuritic growth. B: same explant as in A showing a field of neurites. (326 \times magnification).

TABLE I
NEURITE LENGTHS

Neurite lengths of explants in culture 7 days. V1 and V2 represent two explants from Vitrogen cultures and C1 and C2 are explants on rat tail collagen cultures all from the same culture batch. The neurite lengths, measured from the edge of the explant, are given in mm.

Culture	Length at position:			
	0°/360°	90°	180°	270°
V1	1.45	2.71	1.74	2.42
V2	1.06	1.21	0.00	1.26
C1	0.30	0.00	0.24	0.39
C2	0.29	0.24	0.37	0.41

in neuronal death that is minimized by the short gelation period for Vit. and subsequently is reflected in neuritic outgrowth. In addition, as Vit. does not present a significant barrier, the increased mechanical stability and support (structural or chemical) provided by this collagen gel may contribute to enhanced neuritic growth.

The Vit. matrix does not significantly impede diffusion as indicated by adult rat muscle fibers, cultured in Vit., which stain with vital dyes, histochemical stains as well as with fluorescent compounds (Grega and Jay, 1983; Johann et al., 1984). Spinal cord explants in Vit. culture for up to 2 1/2 weeks have been added to these adult muscle cultures by securing the excised explant with a drop of Vit. (Johann et al., 1984). In coculture, the explants put out a lush growth of secondary neurites and the cocultures are stable enough for experimental manipulations including repeated solution changes and intracellular recording from muscle fibers (Grega, unpublished). In summary, for relative ease of culturing and enhanced neurite growth, Vitrogen is a preferable alternative to the conventional use of collagen coated plates for culturing cord explants.

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