Distance as a Factor in the Development of Attraction Fields between Growing Tissues in Culture[1]

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A SINGLE EXPLANT OF A TISSUE when cultured *in vitro* in either a fluid medium or a coagulum will usually produce a pattern of growth that is distributed radially about its circumference. It has been demonstrated (*1–3*), however, that when two explants are placed in the same culture, the resultant growth is often organized so as to form a pattern about the axis between the two explants. The term "attraction field" has been used to describe this phenomenon.

FIG. 1. 1: Attraction field photographed with polarized light, showing fibroblasts growing on fibrin strands that were rendered birefringent by the tension on the strands. ×90. 2: Stained preparation of a section cut through the attraction field area and showing the reorientation of the fibrin that composed the plasma clot and the resultant growth pattern. ×16. 3: Parallel growth pattern of cells in the attraction field. ×90.

Examination of the plasma clot in the zone between the explants by means of polarized light revealed a birefringent pattern that resembled the lines of force of a magnetic field (Fig. 1). Exploration of this area with the aid of a micromanipulator revealed that syneresis of the plasma clot had placed the fibrin strands under tension. There was also evidence of an

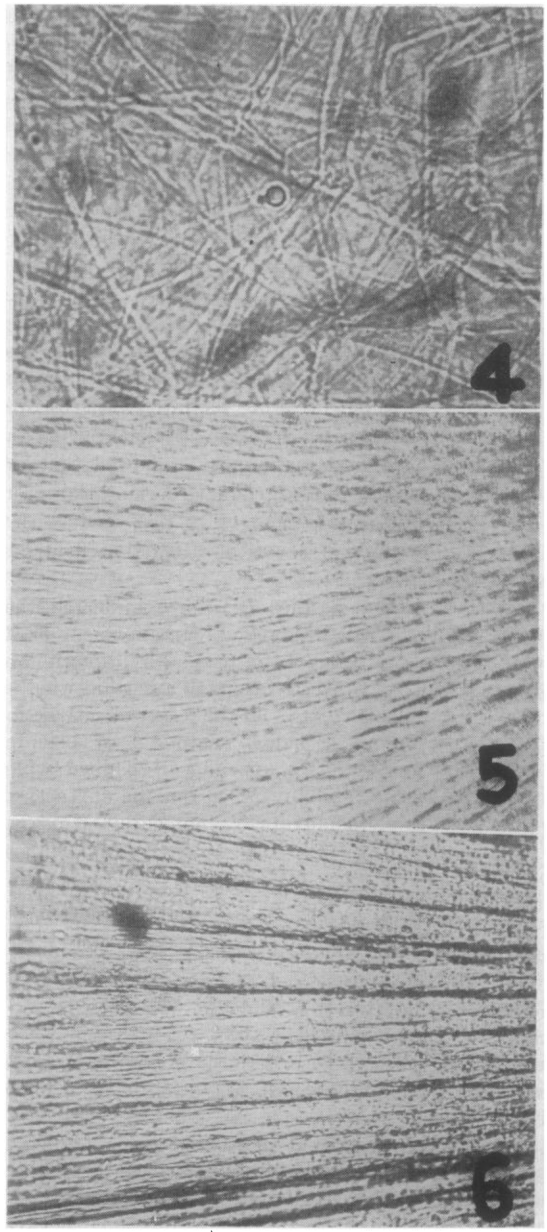

FIG. 2. 4: Fibrin strands in the zone between two explants as they appear in a freshly prepared culture. ×430. 5: Fibrin strands in the same zone, photographed after 16 hr incubation, showing some orientation. ×430. 6: Fibrin strands showing development of parallel pattern 24 hr after culturing. ×430.

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TABLE 1

Group	Distance in mm (d)	d^2	No. cultures	No. attraction fields	Incidence (I)
1	0.0–0.2	.04	48	48	1.00
2	0.2–0.4	.16	51	49	.96
3	0.4–0.6	.36	50	48	.96
4	0.6–0.8	.64	52	48	.92
5	0.8–1.0	1.00	51	46	.90
6	1.0–1.2	1.44	52	46	.88
7	1.2–1.4	1.96	39	33	.84
8	1.4–1.6	2.56	42	34	.81
9	1.6–1.8	3.24	40	28	.70
10	1.8–2.0	4.00	48	36	.75
11	2.0–2.2	4.84	42	26	.62
12	2.2–2.4	5.76	49	28	.57
13	2.4–2.6	6.76	36	20	.55
14	2.6–2.8	7.84	49	22	.45
15	2.8–3.0	9.00	40	12	.30
16	3.0–3.2	10.24	38	14	.36
17	3.2–3.4	11.56	40	8	.20
18	3.4–3.6	12.96	42	2	.05
19	3.6–3.8	14.44	46	0	.00
20	3.8–4.0	16.00	38	0	.00

adhesive force between the growing fibroblasts and the fibrin, as the cells could not be separated from the fibrin strands without breaking the latter. Microscopic examination of this zone at various periods during the development of the attraction fields showed that the fibrin strands underwent a gradual reorientation from a brush-pile pattern in the freshly prepared culture to an oriented pattern after the attraction field had formed (Fig. 2).

X-ray diffraction studies (*4*) have indicated that tension on fibrin will produce a rearrangement of its molecules into a parallel pattern. No attempt was made in the present investigations to ascertain whether the same conditions exist in the case of attraction fields. However, these fields, composed of oriented fibrin strands, acted as a specialized roadbed which was compatible to, and preferred by, the surfaces of the membranes of the advancing filamentous processes of the proliferating cells. Each tissue explant may be considered to set up a mechanism that acts as a stimulus for the development of an oriented pattern of growth for its own cells, as well as the cells of the other explant that shared in the development of the field. It was also observed that growth apart from the zone of the attraction field was in the majority of cases very much reduced in comparison to the area of radial growth in cultures of single explants. Once the gap between the two explants was completely bridged, growth ceased.

It was also noted that attraction fields tended to develop more frequently in cultures where the distance between the two explants was relatively short. No cultures exhibited fields where the distance between the explants was more than 3.6 mm.

A series of 27 groups of cultures was prepared in which the two explants were embedded at various distances from one another. The tissues were of the hearts of eight-day-old chick embryos, and the medium was a mixture of chicken plasma in chick embryo juice. In each culture the distance between the explants was measured with an ocular micrometer immediately after culturing. Any subsequent development of attraction fields was recorded. The cultures were classified into groups according to the distance between the explants. Table 1 lists all groups between 0 and 4.00 mm; of a total of 893 cultures, 548 developed attraction fields.

The incidence (I) as given in the table was obtained by dividing the number of attraction fields formed by the total number of cultures in that specific group. The results were plotted on a graph using the incidence (I) as the ordinate and the square of the distance (d^2) between the two explants as the abscissa (Fig. 3). The line joining the points that represent

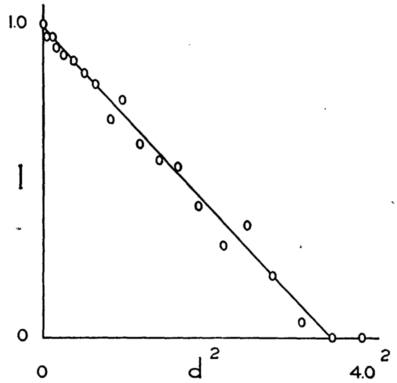

FIG. 3. Graph showing relationship of the incidence of the attraction fields (I) to the square of the distance between the explants (d^2).

groups 6 and 17 forms an approximate mean for all other groups. By employing the loci of these points in the equation

$$\frac{I_1 - I_2}{d_1^2 - d_2^2} = k,$$

we may obtain the slope k, which was found to be $-.0672$. This represents the rate at which the incidence of the attraction fields varies in respect to the square of the distance between the explants.

The deviation (c) of the individual points from this line may be found by employing the equation

$$c = I - d^2k.$$

The values for c very nearly approximate unity.

From these data the conclusion is drawn that the intensity of the forces that initiate the development of the attraction fields for the directional orientation of the proliferating cells varies inversely as the square of the distance between the growing tissues.

References

1. WEIS, P., *et al. Arch. Surg.*, **47**, 425 (1947).
2. GROSSFELD, H. *Arch Entwicklungsmech. Organ.*, **131**, 324 (1934).
3. FARDON, J., *et al. Nature*, **146**, 619 (1940).
4. HERTZOG, R. O. *Naturwissenschaften*, **9**, 320 (1921).