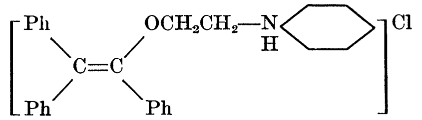
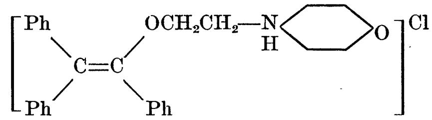
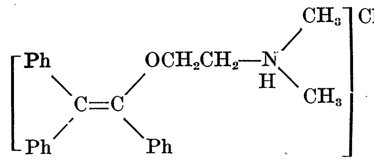
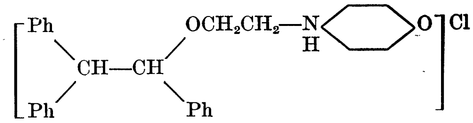
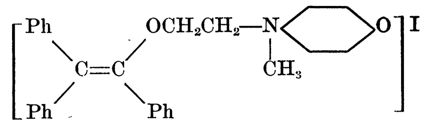
TABLE 1—(*Continued*)

No.	Structural formula	Assayed in	Estrogenic activity in IU/mg
8.		Peanut oil	3000
9.		Peanut oil	100
10.		Peanut oil	200
11.		Aqu. ethanol	< 400
12.		Aqu. ethanol Peanut oil	< 400 < 100

it was not available for testing. The only compound that does not fit this general pattern is the β-piperidinoethyl ether (8). No explanation can at present be offered for its surprisingly high estrogenic potency, which is 15 to 30 times that of the analogously constituted compounds (10) and (9). This was confirmed by several reassays.

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Manuscript received October 15, 1951.

Comments and Communications

"Attraction Fields" between Growing Tissue Cultures[1]

GHOSTS have a way of refusing to be laid. One such ghost is the alleged "attraction" for each other's cells supposedly exerted by two growing parts—for instance, two tissue fragments cultured in a common medium. More than 20 years ago I gave the first description of the striking phenomenon of an oriented cell bridge forming, under certain conditions, between two growing centers (*1*); for convenience this may be referred to as the "two-center effect." At the same time, and repeatedly since (e.g., [*2*]), I have pointed out that the superficial impression of "attractions" (in the customary sense of the word) being at play is a sheer illusion. The correct interpretation, gained by stepwise analysis of the factors involved, has been amply documented and published (*2–5*). It has found even wider currency through the publications of other authors (*6, 7*).

It is somewhat perplexing, therefore, to find in a recent article in SCIENCE (**114**, 431 [1951]) the whole phenomenon rediscovered, redescribed and, by the implications of the terms used, again misinterpreted. In the article in question, entitled "Distance as a Factor in the Development of Attraction Fields between Growing Tissues in Culture," the author, Allan A. Katzberg, states that "the term 'attraction field' has been used to describe this phenomenon." This term is absolutely inappropriate and misleading. Its reaffirmation by the author mars what is otherwise a correct, if not altogether novel, presentation. It matters little that the author is only dimly aware of the systematic work that has been done in this field before. After all, his observations fully confirm the known facts. But the way in which he treats them is apt to lead back to

[1] Work referred to in this paper was aided by the Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago and by a grant-in-aid from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council.

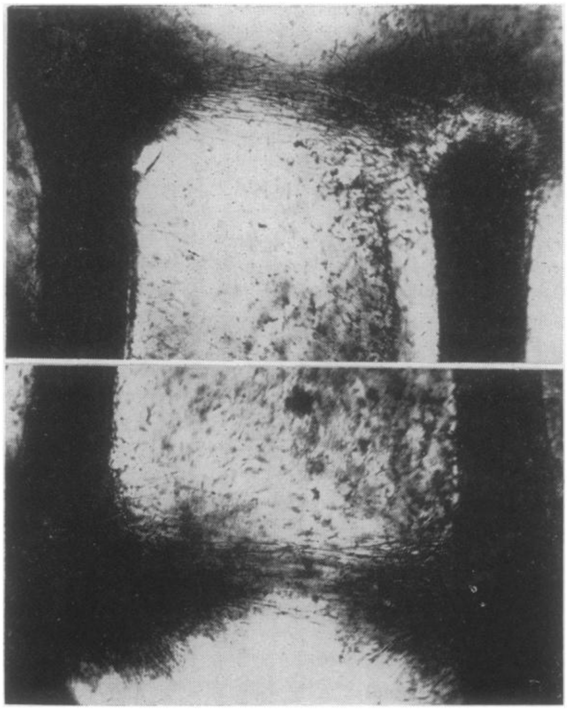

FIG. 1. Two rat nerves, subject to Wallerian degeneration for 17 days, explanted side by side as described in text, fixed after 4½ days *in vitro* and impregnated with silver according to Bodian.

the same old confusion from which our analysis had shown the way out. Therefore, and because of the rather broad implications of the two-center effect for morphogenesis in general, it seems desirable to restate the case briefly.

In order not to be wholly repetitive, I shall use an example not previously published (Fig. 1). Two fragments of adult rat nerve were embedded parallel to each other in a thinly spread clot of fowl blood plasma and embryo extract[2] (thinness of the layer is essential [*2*]). There followed massive outgrowth of sheath cells and fibroblasts from the open nerve ends into the interior of the clot. Near the surface of the clot, however, the cells followed linear, perfectly straight courses reciprocally from one cut end to the other, establishing a cell bridge as striking as in any of our earlier two-center experiments.

As set forth on previous occasions (*1–5, 8, 9*), the mechanism by which this effect is produced has nothing to do with "mutual attraction" but consists of the following sequence of events: (1) The cell masses issuing from the cut ends begin to proliferate, thus constituting local growth centers. (2) Since cell proliferation commonly entails dehydration of the surrounding colloids (*1, 2, 10*), the fibrin around each nerve end undergoes progressive condensation and contraction. (3) These local contractions create tensions in the fibrin net which, for a given amount of proliferation and shrinkage, vary as inverse functions of both volume and thickness of the medium; they are strongest in the surface. (4) For any given medium, the resultant stresses reach a maximum along the connecting line between two foci of contraction. (5) Since the prevailing tensions force the fibrin net into a configuration corresponding to the stress pattern, the fibrin chains, particularly near the surface, are likewise assuming preferential orientation along the connecting line between the two centers. (6) Thus is established a fibrin bridge which acts as a pathway guiding the cells that grow out from either center straight over toward the opposite center. The cells glide over this bridge actively, owing to an affinity for fibrous pathways ("contact guidance"), discussed in greater detail previously (*9*). By no means are they pulled over by traction or lured over by mysterious "attractions."

This, then, is the actual mechanism of the two-center effect. Some of its component steps have, in fact, been correctly recognized by Katzberg; for instance, the condensation and parallel orientation of the fibrin bridge which, in addition to its earlier histological demonstration (*2*, Fig. 8), has now been made visible by polarized light. His main thesis, the decline of the two-center effect with increasing distance between the centers is, of course, self-evident from a consideration of the mechanics of the effect. By consulting an appropriate model (*2*, Fig. 5), it is found that the stress produced by the contraction of the two centers, which is the force aligning the fibrin micellae, is some inverse function of their distance. Just what function, it is difficult to deduce. In an extreme condition, with only a narrow and laterally unattached strip of plasma connecting the two centers, and on the assumption of ideal elasticity of the fibrin net, the linear deformation that is due to a standard degree of contraction c would (according to Hooke's law) be proportional to $\frac{2c}{x}$, where x is the distance. In the actual situation, however, this value will be smaller, because of the continuity of the connecting strip with the rest of the elastic clot, which takes up some of the resulting stresses; further complications arise from the presumable slippage of the stretched fibrin strands, invalidating the applicability of Hooke's law, and the change in mechanical and physicochemical properties of the fibrin chains once they have become aligned and aggregated into larger bundles.

That under these complex conditions the above function should assume the form of exactly an inverse square relation, as reported by Katzberg, seems highly improbable except by coincidence. Moreover, Katzberg's circuitous derivation of this formula from statistical data on the "incidence" of "attraction fields" as a function of distance is rather objectionable, for the two-center effect is basically not an all-or-none condition of "incidence," but a matter of degree. It is true that, as I pointed out in my first report (*1*), the effect has an element of self-reinforcement, so that once initiated it tends to become ever more accentuated as growth proceeds. Even so, there are too many other

[2] This case is taken from a series of experiments done in 1944 with the excellent technical assistance of Hsi Wang.

relevant variables in the picture (e.g., thinness of clot, adhesiveness between clot and cover slip, nearness of culture to surface, meniscus formation, etc.) to put any great stock in a precise quantitative evaluation of the figures of observed "incidence." They do, of course, qualitatively express the general predictable decline of the two-center effect with distance and in this sense are a welcome addition to our knowledge.

These comments should not be construed as discouragement of a more quantitative study of the two-center effect. Indeed, such a study would be highly desirable. But it should be undertaken with a sense of realism—that is, with the nature of the operative mechanism clearly in mind. Perhaps the main danger of a superficial and purely formal treatment is that, if it happens to turn up such empirical data as Katzberg's inverse-square-of-distance relation, and if this is then reported as the property of an "attraction" field, all those unfamiliar with the real situation will jump to the conclusion that this connotes a simple interaction after the fashion of Coulomb attractions. My remarks here are intended to point up the fallacy of such a conclusion. In a wider sense, they may also be taken as a more general plea for greater caution against the rapidly growing indulgence in gross oversimplification in some branches of contemporary biology.

PAUL WEISS

Department of Zoology
University of Chicago

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A FEELING of pleasant surprise was experienced by this writer when he learned that his modest article entitled "Distance as a Factor in the Development of Attraction Fields between Growing Tissues in Culture" (SCIENCE, **114**, 431 [1951]) has attracted the attention of so eminent a scientist as Paul Weiss, upon whom this writer looks as the dean of that field of biology related to growth.

The comments made by Dr. Weiss in the accompanying article are very interesting. However, clarification of some statements is in order. For example, we read: "The author is only dimly aware of the systematic work that has been done in this field." The writer feels certain that Dr. Weiss does not sincerely mean this statement, because he has no data regarding this writer's reading or studying preferences. As a matter of fact, the writer went through numerous references in relation to this question of "attraction fields," "tension fields," "two-center effect," or whatever term was employed in reference to this phenomenon (*1–9*), and he holds views on the sequence of events in the development of the mechanism identical with those expressed by Dr. Weiss. In fact, once discovered in the literature, both German and English, the writings of Dr. Weiss were placed at the top of the list as a "must." Although, unfortunately, not all the journals have always been available, most of his articles dealing specifically with this subject were studied and thoroughly enjoyed.

Certainly the term "attraction field" does not completely describe the phenomenon, but neither does the phrase "two-center effect," which the writer does not recall as having been used in the literature. So, rather than employ the dubious practice of coining a new name, the term "attraction field" was used. Judging from the many favorable comments received since the article was published, the majority, including those not entirely familiar with the situation, were not misled by the term employed, and no other misinterpretations developed.

A short article of this nature does not permit lengthy historical reviews of previous work or of the precise techniques employed, and so the description of the development of this complex mechanism was compressed into one or two short sentences. For instance, the sequence of events entailed in the statement "Each tissue explant may be considered to set up a mechanism that acts as a stimulus for the 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" can be superimposed on the more lengthy outline of events tabulated by Dr. Weiss. Certainly the growing explants induce syneresis of the fibrin matrix which, in turn, produces tension that reaches its maximum in the common axis between the explants, thus establishing a preferred and oriented pathway for the proliferating cells of both explants. No other explanation was considered, and no inferences were made to a possible "mutual attraction" or that the cells were "pulled over by traction or lured over by mysterious attractions." These two phrases not only are entirely foreign to the article but definitely distort the purpose of it.

If, as Dr. Weiss claims in his letter, "By no means are they pulled over by traction or lured over by mysterious 'attractions'," why do we read in his fascinating chapter on "Differential Growth" (*10*), under the topic of "Orientation of Growth," in which the orientation of growth between two centers of contraction is discussed, the following statements (p. 176):

> By its radial orientations such a contraction pattern can evidently guide peripheral cells toward the center. This is one of the major mechanisms by which cells *are drawn* toward distant destinations, as if *"attracted."* [And in the next paragraph], tension is not the only agent capable of orienting tissue structure. Any other physical force that is capable of affecting the orientation and aggregation of polar molecules (electrostatic fields, electrophoresis, streaming, etc.) may have comparable effects.

It was specifically this section of the chapter that made this writer especially cautious and on guard when he drafted the recently published article, so as not to include words or phrases that could be misinterpreted and that would present impressions for which he had no data. And nowhere in the article was it intimated that this was a new discovery of the phenomenon. That was clarified in the first paragraph.

Undue credit is given this author for demonstrating the phenomenon by polarized light. This technique was obtained from previous publications (*6, 7*).

The sole purpose of the article was to show the relationship of the distance between the explants to the frequency of the development of "attraction fields" or "two-center effects." The criticism of the analysis of the data by Dr. Weiss was more than welcome, as was his agreement that the quantitative evaluation of the figures of observed incidence expresses the general predictable decline of the "two-center effect" with distance, and that this is a welcome addition to our knowledge.

ALLAN A. KATZBERG
Department of Histology and Embryology
The University of Oklahoma, Oklahoma City

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Root-Grafting in Tropical Trees

YEARS ago I wrote a paper (*Am. J. Botany*, **21**, 121 [1934]) calling attention to the enormous frequency of natural root grafts in *Pinus strobus, P. resinosa,* and *Thuja occidentalis.* Since then I have noted an almost equal amount of root-grafting in *Acer saccharum* and *Ulmus americana,* and a great deal in many other genera. Kuntz and Riker (*Wisconsin Agri. Exp. Sta. Bull. 9* [1950]) have shown that there are enough root grafts in oaks to allow rapid dissemination of oak-wilt disease through them.

Recently, on the grounds of the Federal Experiment Station at Mayagüez, Puerto Rico, and in the surrounding region, I noted the great amount of grafting in the roots of the mango, *Mangifera indica,* which is very common along the roadsides. Soil wash and road cuts have exposed the roots of thousands of these trees.

Ficus nitida, which is rather commonly planted, also shows thousands of grafts for every tree. Of course, the genus *Ficus* is noted for root production and root-grafting, and the development of strangling figs, so notable a feature of tropical forests, depends on the ready grafting of roots and branches that soon encase the supporting tree in a complete wooden jacket from which there is no escape. Members of the Clusiaceae as well, though not figs, are stranglers and show the same ease of grafting as the figs themselves.

After I had seen the condition of the mango roots, I looked for natural grafts in all of the exposed roots I could find (Table 1).

TABLE 1

Genus	Family	Genus	Family
Albizzia	Leguminoseae	*Inga*	Leguminoseae
Aleurites	Euphorbiaceae	*Mangifera*	Anacardiaceae
Antonia	Clusiaceae	*Manihot*	Euphorbiaceae
Artocarpus	Moraceae	*Maximilianea*	Bixaceae
Casuarina	Casuarinaceae	*Nephelium*	Sapindaceae
Cecropia	Moraceae	*Ochroma*	Bombacaceae
Ceiba	Bombacaceae	*Parkeria*	Leguminoseae
Citrus	Rutaceae	*Delonix*	"
Coffea	Rubiaceae	*Posoqueria*	Rubiaceae
Couroupita	Lecythidaceae	*Pterocarpus*	Leguminoseae
Diospyros	Ebenaceae	*Sapindus*	Sapindaceae
Enterolobium	Leguminoseae	*Spathodea*	Bignoniaceae
		Swietenia	Meliaceae
Erythrina	"	*Tabebuia*	Bignoniaceae
Euphoria	Sapindaceae	*Tectona*	Verbenaceae
Ficus	Moraceae	*Terminalia*	Terminaliaceae
Genipa	Rubiaceae	*Triplaris*	Polygonaceae
Hura	Euphorbiaceae		

In a cursory examination, root grafts were found in 34 genera belonging to 18 different families. Grafts were seen on trees of a number of genera that I could not identify, and many known genera did not show root exposure enough to determine whether root grafts were present. From my observations I conclude that root-grafting is common in tropical trees—perhaps more common than in those of temperate regions.

CARL D. LARUE
Department of Botany, University of Michigan

Separation of Free-Living Cells

DR. NORTHCRAFT'S ingenious use of ammonium oxalate to separate free-living cells from carrot tissue culture provides workers in this field with a valuable tool with which to attack the problem of single cell division (SCIENCE, 113, 407 [1951]). The difficulties he records in obtaining an effective but noninjurious concentration reminded me of similar difficulties that I encountered during certain experiments with ammonium oxalate, to bring about chemical changes in the epidermal cell walls of living cabbage roots (*New Phytologist*, 34, 30 [1935]). It would be interesting if workers with tissue cultures could cause the separated cells to form a solid tissue again. In this connection I suggest replacing the ammonium oxalate in the culture medium by a slightly alkaline solution of a calcium salt.

ROBERT G. H. CORMACK
Department of Botany, University of Alberta