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ASPECTS OF AGGREGATION IN CELLULAR SLIME MOULDS

1. Orientation and Chemotaxis

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In all spheres of biology we are concerned with the orientation of units—be they molecules, cells, organisms, or groups of these—in relation to other similar units and to the general environment, including larger units. We have first to describe their orientation as a behavioral response and then to account for it in terms of their other properties.

At the lowest level, we find that a molecule can influence the orientation of its close neighbors by means of various intermolecular forces, and they in their turn can do the same to theirs. The resultant two- and three-dimensional arrays are ubiquitous in protoplasm and its derivatives (Frey-Wyssling 1953). Their development has been invoked to explain cell differentiation (Weiss 1947); and it has been suggested (Wigglesworth 1948) that even a whole multicellular organism may be usefully considered to be a giant 'molecule'.

As molecules outside such an array (or the electric field commonly associated with biological systems) move at random, their individual paths cannot convey any directional information. Yet specific molecules can influence the orientation of a larger unit in various ways. If they are associated with a solid substratum, they may mark out a path, which should not be much wider than the unit that uses it, unless additional guidance is available; or they may label some fraction of existing pathways. They may be bound to the substratum so intimately that they may to some extent constitute an array, as in specific guides for nerve-fibre growth, and theoretically their orientation could give the path polarity. Or their association may be much looser, as in a scent trail. This could be polarized by a gradient along it, determined by the relative speeds of laying and disappearance; but if it were far outweighed by local differences in deposition, absorption, and loss, there would be no advantage in the pursuer's being sensitive enough to detect it. A hound running back on the scent reminds us that in the absence of polarization (or alternative guidance) a respondent has no means of telling which way to move along the path.

If the molecules are free in a fluid, they can produce an effect in one of two ways. Firstly, in their presence, the unit may respond differently to any other guidance mechanism, of which a current is a rather special example, because it itself may carry the molecules, and indeed may do so for distances much greater than those over which diffusion gradients can effec-

tively operate. "Without convection currents, either spontaneous or self-induced, it is unlikely that insects could orient to odors at all"—Dethier (1947). Sensitivity can in theory be increased till a single molecule is able to initiate a response; and this ultimate limit must actually be approached in the case of some male moths.

Secondly, there may be differences in concentration, which may lead to unoriented accumulation by influencing the speed of movement or, if the responding unit has a certain ability to adapt to the stimulus, the total rate of undirected turning. Orientation—chemotaxis and chemotropism proper—can occur in a gradient that is flat in relation to the unit's powers of discrimination, if the unit has some form of memory and, on the basis of the concentration differences it experiences, is able to choose between the undirected sampling turns it has made. For guided turning, the gradient must be steep enough for different parts of the unit to be stimulated differentially (Fraenkel and Gunn, 1940). Regardless of the sensitivity of the response mechanism, the significance of the local distribution of the molecules must decrease as the differentials and the absolute numbers available for judgement fall.

The chemical can show all degrees of specificity upwards from that of the hydrogen ion, through simple metabolic products and injurious substances, to molecules that are uniquely involved in the response of a single species of unit.

SLIME-MOULD AGGREGATION

Because of the inherent limitations of chemotaxis and the technical difficulties of deciding whether it is involved in accumulation at specific points, its biological importance has been alternately exaggerated and dismissed. But it is not my purpose here to assess the extent to which biological orientation in general depends on chemical gradients; instead I shall consider the problems one group of organisms, the cellular slime moulds or Acrasiales, has faced in relying on such a mechanism.

In the family Dictyosteliaceae, which shows the most interesting life cycle (Olive 1902; Raper 1940a, b, 1941; Bonner 1944a), there are two genera, Dictyostelium and Polysphondylium, available to us for study. During the vegetative phase, their amoebae lead a solitary existence; they feed on bacteria, divide and move independently. In culture they can be maintained indefinitely in this phase (Potts 1902). Some time after the available food has been consumed, they begin to co-operate in their hundreds, and hundreds of thousands, in the erection of aerial fruiting bodies. As a first step in this process, some of the amoebae begin to act as collecting centers; and the others are attracted towards them, joining up as they go to form approximately radial streams, which grow coarser as they age and the finer branches flow into one another. At no time is a plasmodium formed: all the cells remain distinct. After aggregation, the resultant cell masses elongate and in time are borne up into the air on the stalks they

make by invagination of the leading cells; the remaining cells differentiate into spores.

The problem of why amoebae cease to move at random and aggregate towards centres has interested many workers (See Shaffer 1956a); but Bonner (1947) was the first to produce convincing evidence that they oriented to a gradient of a chemical, which he named *acrasin*.

Amoebae able to respond to centers will not move towards blocks of agar on which even large numbers of them have rested for varying periods, nor will they collect at the tips of capillary tubes containing various extracts of centres or the water that has bathed them (Shaffer 1953). It may be concluded either that a center imposes some further characteristics on the chemical signal or that secreted acrasin rapidly becomes inactive; if it were merely volatile, one might expect to get positive reactions to the capillary tubes, as did Cooke, Elvidge and Heilbron (1948) working with Fucus sperm.

Would the organism gain any advantage by inactivating it? If we assume that however great the absolute concentration is made there is a certain minimum percentage difference an amoeba can detect, the range from which it could be attracted to a point source of stable chemical in a large volume of medium would be strictly limited, as the steady-state relative concentration gradient would not be influenced by the amount of chemical secreted. But if the chemical were inactivated in a suitable way, the relative gradient at any distance could be increased, though the concentration would be lowered. The more rapid the inactivation, the greater the effect. A source could thus increase its range indefinitely, in theory; but in practice the limit would soon be reached, because the price of having to secrete more chemical, and of having to wait longer before the cells furthest away responded, would rise so enormously as the range was extended.

If the medium is actually limited in extent, or if it is effectively limited by the presence of other sources, inactivation is essential if a source is to maintain any range at all for long periods: this is simply a question of checking the growth of the background against which the gradation has to be detected. This need can be visualized by dropping a particle of dye into still water: one finds it progressively more difficult to judge where the particle is by examining, through a mask, part of the area coloured by diffusion; and the smaller the volume of liquid, the more rapidly does this happen.

Slime moulds can in fact aggregate on a bare glass surface in a damp atmosphere, in which conditions the volume of medium must be extremely small. Further, under even a shallow layer of water they are prevented from building fruiting bodies (Potts 1902), and instead they go through alternate phases of aggregation and dispersion for several days in the same medium. All this suggests that the secreted chemical is inactivated.

It may be noted, though, that if the reacting cells can remain oriented for some time after they have temporarily ceased to receive positive guidance from a chemical gradient, a source can maintain effective guidance

with a *stable* chemical for longer periods if it releases it not at a constant rate but in pulses at appropriate intervals. Even if the chemical were impermanent, it would be more efficient to do this. The possibility of there being such pulses in aggregation is brought to mind by the time-lapse films of this process taken by Arndt (1929, 1937) and Bonner (1944b), which show waves of rapid inward movement spreading outward from the centre, although Bonner's film and others taken by myself make it clear that such waves are not always present.

Whether the chemical is inactivated or fluctuation of the gradient is essential for stimulation, amoebae sensitive to the chemical should be able to orient to an artificial source that can be renewed at frequent intervals with the liquid obtained from the immediate vicinity of a natural source only a few seconds before. If a drop of this liquid, freed from cells, is added all round the edge of a block of agar a few millimeters square, resting on a glass slide, any acrasin it contains can reach amoebae sandwiched between the block and the slide only by diffusion; and fresh liquid can be added without mechanically disturbing the existing gradient. When this is done every minute, it is found that after about 10 minutes, the sensitive amoebae have turned, elongated, and begun to move more or less perpendicularly towards the nearest edge of the block (Shaffer 1953). Those close to a corner adopt an intermediate orientation and so form a pattern not unlike a quadrant of a natural radiate aggregation, but inside out—the artificial 'center' being at the periphery.

Acrasin solution as collected, even if cell-free, is inactivated within a few minutes at room temperature, due to the presence of an extracellular protein. If this is removed, acrasin is remarkably stable (Shaffer 1956a). The addition of a drop of its solution to the edge of a test agar block but once—and so a single acrasin pulse—is sufficient to bring about the orientation of the amoebae in two to three minutes (Fig. 1). As there are unlikely to be separate sensory, transmitting, and effector systems, it is probable that acrasin acts directly on the cell surface at the front end of an amoeba and causes a differential outflow of cytoplasm.

The following analysis is based on a recent series of papers (Shaffer 1956 a-d). Even at the periphery of an aggregation several centimeters across, an amoeba must be able to detect a difference in concentration at points only about 10 μ apart. Whatever its sensitivity and however acrasin were inactivated, far less time and chemical would be needed to establish an adequate gradient if the center were not the sole secretor but were helped by a series of boosters. In fact, as Bonner (1949) concluded from the observation that amoebae did not crawl straight towards the center but condensed into streams on their way there, streams do secrete acrasin. What has to be explained now is what ensures that the amoebae reach the center at all, or in other words, what makes the boosters carry the center's message rather than their own.

It has been assumed in the past (Arndt 1937; Bonner 1949; Sussman 1954) that the center controls aggregation (i) because of the number of cells accumulated there and/or (ii) because these cells are specially active in producing the attractant. Let us consider the first explanation. Amoebae before aggregation move at random; and it has been suggested that if they then all began to make acrasin, centers would develop where they happened locally to be closer together. However, if all of them did suddenly start to secrete at about the same concentration, one would expect them to form small clumps all over the field, which would grow by irregular fusion till they were out of range of one another. This would be something like gravitational condensation, though it would be modified by the cells being polarized and by their adhering on contact which would tend to limit their direction of movement, and by their ability to change

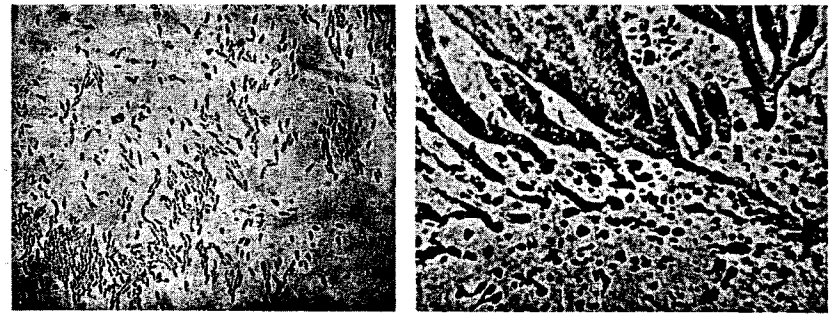


FIGURE 1. Amoebae of *Dictyostelium discoideum* lying under a block of agar and moving 'downwards' to the nearest edge of the block. 3 minutes after adding a drop of concentrated acrasin solution to the edge. × 80.

FIGURE 2. Amoebae of *Dictyostelium discoideum* streaming from an area where the cell density is high towards a centre (extreme right) that has formed where it is low. × 35.

speed, so that if one clump came to rest it would collect the others in the neighbourhood. But aggregation is not like this: the cells move towards the centre, broadly speaking, along radial pathways.

Further, if the food supply is unevenly distributed, the cells in an area where there is much available will multiply extensively; but they may find when they are ready to aggregate, that the center to which they are attracted has already formed in a neighboring sparser area. If this small center's influence depends on its size alone, one cannot explain how it remains the goal of streams that become thickest at their outer ends and taper steadily towards it (Fig. 2).

It might be supposed then that for the center to have much greater powers of secretion per cell than the periphery is both necessary and sufficient for aggregation to be radial. Actually it is neither. The influence of the chemical released by the center must fall off rapidly with increasing distance simply because of the shape of the diffusion gradient; and it must be so much further devalued by the output of the boosters, and by the higher

degree of inactivation their use demands, that it cannot possibly directly dominate the whole of an aggregation. As there must then be some other basis for centripetal movement in the outer part, one may suppose that the same mechanism is at work nearer the center, an area of much the same appearance. In short, if the center's secretion cannot dominate the gradient throughout an aggregation, there would seem to be no advantage in its dominating it anywhere except in the center's immediate vicinity. Thus, there is no point in the center's producing continuously or intermittently a much higher concentration than the periphery ever does (unless its inactivated acrasin is essential to the latter as a raw material—an improbable biochemical specialization). In fact, when there are no cells intervening, a center can attract cells from only about the same distance as a stream can—a mere few hundred micra—and direct comparison by Bonner's (1949) method shows that a center and a stream do secrete acrasin at something like the same concentration.

However, if the concentration at the center were fractionally greater than that in the part of the stream adjacent to it—a difference perhaps too small for his method to detect—and if this difference were continued out along the stream as a gradient of secretion, controlled so that it were not for most of the way far steeper than the amoebae needed, this could well provide guidance for much greater distances than could a gradient in the external medium. Yet, using another of Bonner's (1949) methods, no gradient in the amount of acrasin escaping from the sides of a stream is detectable; moreover, cells in a continuous compact stream do not usually turn towards a center transplanted close beside it.

Still, gradient guidance could operate even if the locus of highest concentration were not the center: if zones of temporarily increased acrasin secretion spread centrifugally along the streams—as is likely involved in producing rhythmic movement—they could expose the cells to adequate gradients as they reached them; and the reverse gradients after they had passed would not necessarily have an equal and opposite effect. But though perhaps such pulses do sometimes play a part in keeping stream cells oriented, they are probably neither essential nor constantly present. The absence of photographically recordable rhythms from some aggregations suggests this, though it is not conclusive. There is also the observation that when amoebae of one member of certain pairs of species are attracted to a stream made by the other member, the separate stream that they form alongside it, because surface differences prevent them from coalescing with it, can flow in either direction. Thus, when the first foreign cells reach the original stream, they cannot experience an acrasin gradient, of any kind, adequate to make them turn towards the center, though, of course, their sensitivity may be different from that of the cells in this stream. It is most likely that after these first foreign arrivals have made an undirected turn, the later ones adopt the same orientation by a contact reaction.

Whereas the separate cells before they have entered an aggregation do not secrete acrasin and have such a low degree of intercellular stickiness

that, in most species, they separate freely again after they have come in contact, the cells in streams and centers do secrete and are highly sticky—a property that is the basis for the mechanical strength of these structures. If it is assumed that the stimulus that induces the first type of cell to develop the properties of the second—a change that can be called integration—is contact with the second type, it is possible to explain why aggregation should be radiate even in the absence of quantitative dominance by the center. Cells that initiate centers by secreting acrasin would attract the neighboring cells towards them; only when these had made contact with them and founded the streams would they begin to secrete and attract the cells beyond them, and only when these had stuck on to the incipient streams and were guided by the flow therein rather than by the chemical gradient would they in their turn begin to secrete.

The development of aggregations in *Polysphondylium violaceum* appears to bear out this scheme. Centers are first visible as small clumps without any streams, and the latter then extend progressively outwards from them. Usually no streams form that are not from the beginning joined to the main system. Still, this does not prove that a cell must make contact with integrated cells for it itself to become integrated: it might be that it is stimulated to do so at some distance from the stream or center to which it is moving, but that it takes as long to change as it does to reach it. Though this may seem to be an unnecessarily complicated arrangement, it has been found to be operative. If the unintegrated cells are attracted towards a center that is dragged away as they move towards it, they will in due course form a compact, sticky, acrasin-secreting stream, without ever making contact with it. It is acrasin itself that induces integration.

Bonner derived the name *acrasin* from the Acrasiales, but he also had in mind the witch Acrasia in *The Faerie Queene* (Spenser 1590). In the Bowre of Blisse was to be found this

> *. . . . faire Witch her selfe now solacing,*
> *With a new Lover, whom through Sorceree*
> *And witchcraft, she from farre did thether bring.*

She was by no means content with merely attracting travellers; and after she had had her way with them, the untutored eye failed to recognize her hangers-on for what they had been:

> *. . . . these seeming beasts are men indeed,*
> *Whom this Enchauntresse hath transformed thus.*

It is thus most apt that acrasin has now been found not only to attract cells but also to alter their shape, surface, and other properties. It would be yet more fitting if sexual union were the basis of aggregation, as Wilson (1952, 1953) has indeed claimed; but so many workers on these organisms (Sussman 1954; Shaffer 1956d; see Wilson for earlier references) have failed to obtain evidence for this that we should scarcely be justified in likening the culture plate to the Bowre of Blisse.

Although contact is not the stimulus for integration, in *P. violaceum* the relationship between the distance at which the actual stimulus is re-

ceived, the time taken for this change to happen, and the speed of movement, is such that in the absence of experimental interference the cells will usually have reached the stream and be subject to flow guidance before they begin to secrete. This still provides an adequate explanation of why aggregation should be radial. However, it is common in some other species, including *Dictyostelium discoideum,* for an aggregation when it first appears to consist not of continuous compact streams but of diffuse expanses of cells, many of which are oriented though they are not in contact. From the extent of these 'stippled' aggregations, and from the sweep of the stream areas that results in the general orientation being in places directly away from the center, it is clear once again that the center is not even the main acrasin secretor. Yet though they are all releasing acrasin, the separate cells within the aggregation do not, by and large, unless they are disturbed, turn towards the cells beside them: they tend to remain pointing in the direction from which orientation spread and eventually to condense into definitive streams that are radial. The explanation appears to be that the effect of orientation, a process that involves turning and elongation, is, on average, to bring the front end of a cell closer to the cell that first attracted it than to any other; and only after this has happened does it secrete maximally. If the resultant attraction then acted through the centers of the cells, as it would do if aggregation were gravitational, it would not show a directional bias. It is because the gradient is measured at the front end that there is a higher probability that this resultant will be in the direction from which orientation spread rather than another. Closer examination of such an aggregation shows that orientation is only in general radial and that local divergence is common. That orderly aggregation does depend on the time relationship between orientation and secretion is shown by disturbing this experimentally: aggregation then becomes chaotic.

Though nothing is yet known about the pattern of emission by a single amoeba, it may be pointed out that if it released acrasin at the same concentration all over its surface, there would be a bigger gradient leading up to its highly curved rear end than to its sides, simply because of its shape. This difference might be of importance to the cells of low sensitivity in an early aggregation and be one of the factors helping them to join up end to end. Similarly, diffusion cannot produce anything like as great a relative concentration gradient near the surface of an aggregate of a few thousand cells as it can near a single secreting cell or a small clump of them. This may explain the observation that cells just beginning to turn into a stippled aggregation, and therefore guided by acrasin, may yet not respond to an aggregated cell mass that happens to migrate over them; though perhaps half an hour later, and especially if they are first disoriented, they will do so. Paradoxically, for a brief period while sensitivity develops, it may be that a source can be too big to be attractive.

It is suggested that centers owe their position not to their constantly secreting *more* than the peripheral cells but to secreting *before* them; and

this is due primarily to the ability of the cells that initiate the centers to release acrasin into the surrounding medium when there is either none there or not enough to bring about orientation. But their position is not then completely assured, and especially in the early stages of some species, a field of amoebae may successively enter different organizations (Arndt 1937; Raper 1940a). Arndt attributed this to changes in strength of the stimuli produced by the centers.

Probably these reorientations are best considered as special cases of rhythmic activity. In this, zones where the cells are moving more rapidly towards the center spread out from it. In one very slow and coarse form—the 'fairy ring' pattern of *P. violaceum* (Shaffer 1956d)—the cells between the fast zones actually lose their integration and wander about at random. The corresponding cells in the quicker and more delicate rhythms sometimes revealed by time-lapse films suffer a less extreme change of state, the only visible alteration being in their speed of movement. That these rhythms may embrace cells that are not in contact makes it probable that acrasin plays a part in their generation; but it is not yet possible to choose between the numerous hypotheses that would account for them, because the temporal course of a cell's secretion and velocity changes, if any, in response to acrasin are unknown. By using acrasin solutions to provide stimuli of known characteristics, one should be able to obtain the necessary information. For the present, it seems clear that when cells are moving alternately quickly and slowly in time with their neighbors, they are stimulated, discharge, recover, and then are stimulated again. It might be, for example, that acrasin reaching the external surface of a cell alters it so that there follows a greater outflow of both cytoplasm and acrasin. The subsequent slowing down must be due to fatigue or adaptation of one or more of the receptor, secretory, or motor systems (in so far as they are separable). In a stippled aggregation, in which the cells cannot yet avail themselves of flow guidance, it must be especially useful for the center to be able to reinforce its authority by repetitively re-exciting to some degree the acrasin relay system—'adaptation', among other factors, presumably helping to reduce the effect of the reversed conditions at the back of each wave. It is not known whether the physiological differences involved in initiating aggregation persist and play any part in pacemaking.

The strength of a relayed wave at any point depends presumably on the responsiveness of the cells locally, and also on the stimulus they receive, and so on the strength of the wave since its inception. Two opposed waves able to command about equal responses should bar each other's way; but quite a small difference in their strengths at the site of collision might ensure the survival of one of them. If a new wave started from a center before that from a neighboring one, it might be able to exploit a purely temporal advantage and, if they had effectively recovered, reorient the cells beyond the former aggregation boundary. As soon as a gap developed between adjacent organizations, there would be no further possibility of their interaction, had it existed. Examination of Arndt's film of *D. mucoroides*

does show that in early aggregation a wave may invade an area previously conquered from another direction and that waves may or may not cancel on collision.

A ring may form at the forward end of an isolated length of stream if the cells in the lead are attracted back to one side by the cells behind (Shaffer (1956c). On the other hand, a circularly polarized organization sometimes develops while the cells are still separate from one another (Raper 1941). This may arise if a wave is weakened in some area of critical size, or is blocked completely, but is able to cross it in the opposite direction, after spreading round it, as a result of a local increase in strength or a change in responsiveness of the cells within the area, provided that the wave reaches the side it first approached, and so completes the vortex, after the cells there have become ready to respond again and before a new wave (if there was to be one) has arrived. Whether the wave continues to circulate or not, the cells will then have to pursue ever-receding pursuers.

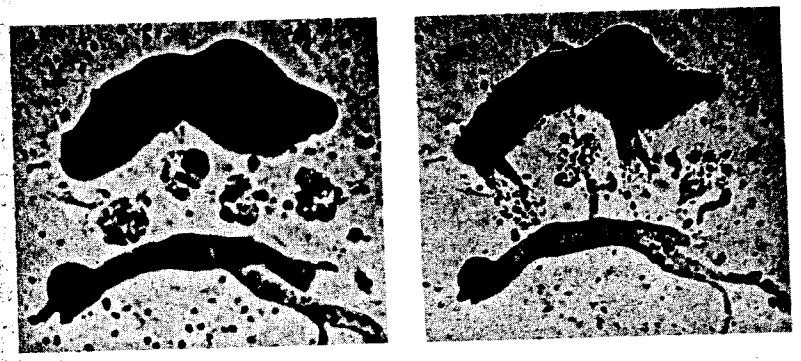
It may be mentioned that in addition to the waves we have been considering, of which at most one or two travel across an aggregation at the same time, there may be very much finer ones. These 'ripples' on the streams, only a few amoeba-lengths between their crests, are revealed most beautifully by Arndt's film. As far as one can see, unlike those of the first kind, they do not cross gaps between cells, and there is at the moment no reason to suppose that they are caused by rhythms of acrasin secretion; perhaps they are of mechanical origin.

SPECIES SPECIFICITY

Raper and Thom (1941) found that mixed amoebae of any two of the species *D. discoideum, D. mucoroides,* and *D. purpureum* would enter joint aggregations, whereas any of these Dictyostelia would form aggregations that were distinct from those made by *Polysphondylium violaceum* in the same area, the streams even overlapping. They further observed that common aggregates of *D. discoideum* and *D. purpureum,* whether arising naturally or as a result of manipulation, would always in time separate into their components, unless they were rushed into building fruiting bodies before they had been able to do so. In contrast, those of *D. discoideum* and *P. violaceum* would not associate even temporarily when placed in contact.

One factor involved is the degree to which the cell surfaces of the different species resemble each other. We may explain the results with the mixed Dictyostelia by supposing that the cells of these species are oriented by the same chemical gradient and that their surfaces are sufficiently similar for them to enter common streams and to be subject to the same flow guidance but different enough for them slowly to sort themselves out. Is it possible to account for the development of the *Dictyostelium-Polysphondylium* mixtures mentioned, simply on the basis of greater differences in their cell-surfaces, without invoking two types of acrasin? Certainly the amoebae of *P. pallidum* can be attracted to a *D. minutum* stream, for which they have such little contact affinity that they are not guided by it

on their arrival but are left to wander about by themselves, sticking to those of their own kind when they meet and eventually forming a stream. This remains applied to the side of the *minutum* stream because of the transverse acrasin gradient it experiences; but possibly it might be attracted away from its bound companion and enabled to join up with another of its own species, if there were a similar pair of streams running close by that temporarily or permanently secreted acrasin at a higher concentration. Whether or not joining up occurs in this way, specific aggregations clearly can be produced using only one acrasin.



A B

FIGURE 3. A. A young fruiting body of *Polysphondylium violaceum* (top) has been laid on the agar parallel to a stream of this species, and four heaps of amoebae, taken from aggregations, deposited between them. The first and third from the right are of *violaceum* also: they start rather nearer the fruiting body but move towards the stream (B). The other two heaps are of *Dictyostelium mucoroides*: they start rather nearer the stream out are attracted to the fruiting body. × 35.

But to return to the mixtures used by Raper and Thom—the amoebae of their Dictyostelia when deposited beside a *P. violaceum* stream are not attracted towards it, though they are to one another's streams. It looks as if here there must be two acrasins involved. A further complication is that these Dictyostelia do respond to young fruiting bodies of *P. violaceum* and even to its older centers and to certain parts of its streams that have begun to develop like centres, whereas these masses often have very much less influence over sensitive amoebae of their own species than do the streams. The simplest explanation of this in terms of specific chemicals is that the *violaceum* streams and young centres secrete an acrasin that attracts *violaceum* amoebae, but that the older stages partially or completely switch over to making the acrasin that attracts certain Dictyostelium species (Fig. 3; Shaffer 1953).

There is, however, a very different explanation available, and though some findings make it an improbable one in the present instance, it does

show the possible refinements of a chemotactic system. Theoretically, two types of amoebae could sort themselves out using only one chemical, if they differed sufficiently in their responses. As an extreme example, if one type reacted rather slowly to an acrasin stimulus and adapted (or fatigued) very little, it would maintain the orientation and secretion induced by the spread of the relay initially. If the other one both reacted and adapted very quickly, a field of its cells could transmit a series of frequent short acrasin pulses, which would control *its* orientation. The two types would be able to form independent aggregations in the same area, provided that their surfaces were sufficiently different for them not to interfere with each other's movements on contact and that the peaks of acrasin concentration produced by the second type were higher than the level maintained by the first. Some separation would still be possible with two types of cells whose behavioral responses were much more alike; but the smaller the differences, the less efficient it would be.

To explain some of the results obtained with different *P. violaceum* sources, one might suppose the pattern of the acrasin secretion of the young centres and streams to be such as to excite the response of *violaceum* amoebae and that of the older centers to be increasingly effective on the amoebae of certain Dictyostelia. There is, though, some evidence obtained from *D. discoideum*, the species most studied, that the frequency of pulses is not critical for orienting the cells used experimentally as 'sensitive'. But the cells in an early stippled aggregation may be more exacting. The inadequacy of the acrasin signal provided by an aggregated mass crawling over them possibly may lie not in the magnitude of a static relative concentration gradient, as was suggested before, but in the absence of an appropriate fluctuation. And though pulses crossing gaps between cells are not visible in some films of mine that record the response of mixed populations to specific gradients, it is just conceivable that waves of acrasin secretion might not affect speed.

Whether any of these specificities, and others discovered by examining further species, *are* due to patterns of secretion and response is thus unknown, and cannot be determined by seeing which cells are attracted to which natural sources. The agar-block test should be more useful, when used with purified acrasins, as it enables us to expose the cells to gradient fluctuations of known frequency. Perhaps the problem should be left till the acrasins produced by different species and different stages have been compared as spots on a chromatogram; and then it can be seen whether there is any residual specificity that cannot be ascribed to chemical differences.

THE NERVOUS SYSTEM

It may not be impertinent to point out that a slime-mould amoeba and a developing nerve fibre are faced with much the same problems in reaching a distant goal; and there are considerable similarities in the way they solve them, even though much recent work (e.g. Weiss and Taylor 1944) has

not supported the view that the tip of a growing nerve can be guided to a remote tissue by a gradient of a chemical that this hypothetically produces (Ramon y Cajal 1928).

(1) According to Weiss (summarized 1941, 1955) the tips of the nerves (of vertebrates) that grow out first are guided by contact with structures in their environment, and especially linear fibrils and microfibrils. (2) As different types of pioneering fibers may choose different pathways when presented with alternatives (e.g. Taylor 1944), this initial guidance must be to some extent selective. (3) The way in which the nerve supply is adjusted to the needs of the area innervated is undoubtedly complex (Hamburger and Levi-Montalcini 1950); but it is clear that the pioneering fibers must convey some information; and it has been suggested that the metabolism of a cell-body changes when its fiber has established connection with its end-organ, and that this, perhaps as a result of the release or absorption of diffusible substances, influences the differentiation of neighboring cell-bodies. (4) A pioneering fiber that happens on its appropriate end-organ becomes adhesive for other nerve fibers, and so (5) is able to guide those growing out from its neighbor neurones to their destination, if they apply themselves to it. (6) These further neurones influence the differentiation of the cells next to them, and their fibers in their turn become adhesive and act as guides. (7) This fasciculation is selective, nerves preferring, to a varying extent, to run beside others of the same type (Oppenheimer 1941; Holtzer 1952). (8) If the end-organ shifts its position, the nerve bundle is towed behind it.

In slime-mould aggregation, as we have seen, (1) the amoebae are first guided by chemical gradients in their environment. (2) These gradients are specific for different species. (3) After the amoebae nearest the center have oriented, and in some cases only after they have made contact with it, they begin to release the chemical and so induce more distant amoebae to start differentiating and to move towards them. (4) The first amoebae to be oriented also become adhesive and so (5) can provide contact guidance for others that apply themselves to them. (6) The more recently recruited amoebae become attractive and adhesive in their turn and both guide those still further away and cause them to differentiate. (7) The formation of streams is selective, amoebae of different species joining preferentially, to a varying degree, with others of their own kind. (8) If the mass at the centre begins to migrate, any streams still flowing into it are towed behind it.

With this degree of similarity in the mechanism or orientation, it is not surprising to find the same patterns developing both in nervous systems and aggregations. Thus, a ring may be formed at the leading end of either a nerve fiber (Wigglesworth 1953) or a stream, if the tip happens to turn backwards; and then round and round the nerve must grow or the cells must flow.

The intercellular connections of the central nervous system, as of an aggregation, emerge in a field of cells that are for the most part unoriented. Because of their inaccessibility and indescribable complexity, it has

proved very much more difficult to determine the factors responsible for their development and orientation than those operating on nerve fibers in the rest of the animal. (Fortunately, an aggregation, on a culture plate, is effectively two-dimensional and displays itself for convenient observation.) The minutiae of the pattern of mutual relationships vary from one individual's nervous system to another's (Lashley and Clark 1946) and from aggregation to aggregation and are doubtless without functional significance. All the variant patterns established by a population of cells, with properties and initial distribution defined within certain limits, will belong to one family; and what we should aim to describe is the Highest Common Form and Function its members will share.

There are some resemblances between an aggregation and a *functioning* nervous system; but as so little is understood about the relevant phenomena is slime moulds, it is difficult to assess the usefulness of making the comparison. For example, self-propagating activities of various kinds can spread along a line of amoebae at speeds as fast as 1.0 mm a minute, or a hundred or a thousand times slower; and they may fail to pass cell junctions in some places. Again, the production of a circularly polarized organization during the early stages of an aggregation is rather similar to the establishment of a wave of contraction circulating round a ring cut out of a jellyfish medusa, which, as Bozler (1926) showed, can be started by blocking one of the pair of nerve impulses that travel in opposite directions round the ring from a common point of origin; though, as the amoebae do not give an all-or-none response, with them there is perhaps only a local weakening or strengthening of one arm of the wave, which would correspond more to Mayer's (1906) original explanation of the jellyfish behavior. As changes in the cell surface are central to them, it is not improbable that these activities of slime moulds and the specificities for which some of them possibly are responsible, could usefully be studied by measuring electrical properties.

SUMMARY

The movement of slime-mould amoebae towards certain collecting centers, which have been started by a few of the amoebae, depends basically on their orientation to the maximum gradient of a chemical, acrasin. Because the gradient is produced by diffusion, the direct range of a center must be limited by the size and maximum sensitivity of the responding amoebae, the amount of chemical that can practicably be produced, and the time needed for orientation to occur after secretion has begun. In fact, its range is rapidly and economically extended by a relay system, as acrasin not only orients an amoeba but induces it to become sticky and secrete acrasin. But as a result, there may be a hundred-thousand sources within an aggregation; and the organism meets the problem of maintaining detectable gradients by inactivating secreted acrasin with an extracellular protein. However, this further reduces the importance of any influence the chemical secreted by the centre could have at the periphery. The amoebae move towards the center not because of a sustained gradient of secretion outwards from it, but because of the time sequence in which the relay operates. In one type of aggregation, the delay before an oriented amoeba begins to secrete is sufficient for it first to reach the amoebae that attracted it; and as it can then depend on contact guidance, it is not disturbed by the acrasin production of the amoebae on its flanks and at its rear. The result is that the amoebae, stuck together in radial streams, converge on the point where acrasin secretion began. In another type of aggregation, the delay is less, and the amoebae start to make acrasin before they reach their primary attractors; nevertheless, they too condense into radial streams, because, once the field has been polarized, there is a greater probability that an amoeba will continue to be attracted in the direction in which it has been oriented than in any other. The center owes its position not to constantly secreting more than the peripheral amoebae but to secreting before them; and this is primarily due to the ability of its initiator to release acrasin into the medium without their being any there already. If the time relationships of the relay are seriously disturbed, aggregation becomes chaotic. The secretion of a center may fluctuate, and this may lead to the periodic re-excitation of the relay system. The resultant pulses spreading out over an aggregation may reinforce flow guidance in its streams and are especially useful in repetitively reasserting the authority of the center in an aggregation in which the oriented amoebae are not in contact. Also, acrasin is used more economically if an adequate gradient is maintained for only a fraction of the total time. Various reactions with neighboring relay systems are possible: and peculiarities in the spread of a relay may lead to an aggregation's being circularly polarized. Specificity in aggregation may depend on differences in cellular adhesiveness, the acrasin, or the pulse pattern. There are many resemblances between the orientation of aggregating amoebae and of growing nerve fibers.

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