

### 1.3 Aggregation of Slime Mold Amebae

In this section we shall formulate a mathematical model to describe the aggregation of slime mold amebae. We shall thereby gain some understanding of a phenomenon of considerable current interest in developmental biology. (*Developmental biology* includes *embryology*, the study of how a single cell develops into a complicated embryo, but also encompasses the development of simpler organisms that cannot be said to have an embryo.) We begin with some facts about slime mold amebae that provide a basis for our mathematical model.

#### SOME FACTS ABOUT SLIME MOLD AMEBAE\*

One can begin a description of the life cycle of the slime mold amebae at the spore stage, where each ameba is dormant within a protective covering. When conditions are favorable, an ameba emerges from its spore. Of the order of 10 micrometers ( $10^{-3}$  cm) across, the amebae are rather shapeless one-celled organisms that move by extending contractile portions of themselves (pseudopods).

The natural habitat of the amebae is soil or dung. An important element of the food chain on earth, they feed on bacteria by engulfing them. If food is plentiful, the amebae continually feed and multiply by mitosis (dividing in two). If the food supply becomes exhausted there is an **interphase period** of random and somewhat feeble movement, where the amebae are more or less evenly distributed over the area available to them. During this period, the disappearance of the food supply triggers certain changes in the amebae. The details of these internal changes are not known, but there is no difficulty in observing the striking external phenomenon that results. After a few hours, the amebae begin to aggregate into a number of collection points. Typically,† these are more or less regularly distributed, with a spacing of a few hundred micrometers. (See the frontispiece. Figure 1.4 gives an idea of what the beginnings of aggregation look like under conditions of lower cell density.)

After aggregation has been completed, the amebae that have collected at a given point (ranging in numbers from a few in laboratory experiments, up to 200,000) form a multicelled slug. This moves as a unit, although the formerly free living amebae retain their cell walls within the slug. Then the slug stops and erects a stalk, on top of which is a roundish container of spores. The cycle is thus completed.

\* "There is no philosophy which is not founded upon knowledge of the phenomena, but to get any profit from this knowledge it is absolutely necessary to be a mathematician."—Daniel Bernoulli. Quoted by C. Truesdell on p. 318 of *Essays in the History of Mechanics* (New York: Springer, 1968).

† There are several species of cellular slime mold, with various corresponding differences in behavior. [See J. T. Bonner, *The Cellular Slime Molds* (Princeton, N. J.: Princeton U.P., 1967).] Our description is appropriate for the most studied species, *Dictyostelium discoideum*.

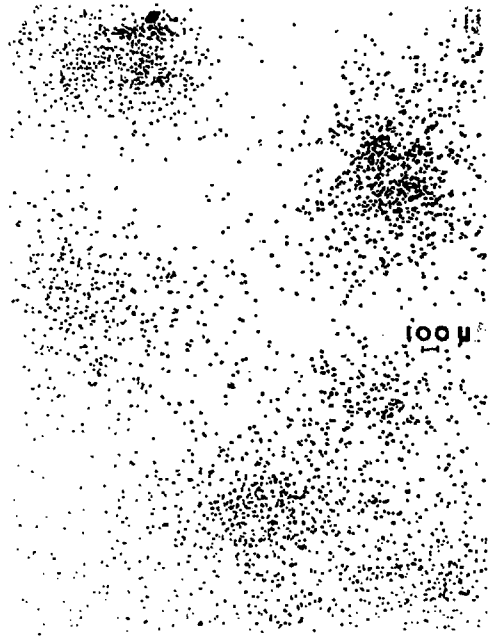


FIGURE 1.4. *Population of amebae just beginning to aggregate. The scale is indicated by a 100-micrometer line segment. (Courtesy of B. Shaffer.)*

What is responsible for the organized aggregation of the amebae? This is an important question, for “purposeful” movements occur frequently in developmental processes. Usually, these movements take place rather inaccessibly within a developing organism, but the cells of the slime mold amebae will obligingly perform in a laboratory dish so that it is easy to examine them and to experiment with them.

It has been discovered that the amebae move preferentially toward relatively high concentrations of a chemical which they themselves secrete. In some species the attractant has recently been identified as cyclic 3',5'-adenosine monophosphate (AMP) a chemical with many important biological functions. (E. W. Sutherland was awarded the Nobel Prize for medicine in 1971 for his work in elucidating some of the roles of cyclic AMP.) It is also known that a given quantity of attractant loses its potency in a matter of minutes. This has been traced to the activity of an enzyme that alters the nature of the AMP.

Presumably, aggregation is caused by the fact that the amebae move up a gradient of attractant, but what determines the time of onset? What determines the spacing of aggregation centers? Can one quantify the process? A mathematical model is needed to answer such questions. We shall proceed to devise the simplest model that could reasonably be supposed to bear on

the circumstances.\* If analysis of this model is encouraging, one can add detail later. For this, the reader is referred to the paper just cited and to subsequent papers by various authors in the same journal.

#### FORMULATION OF A MATHEMATICAL MODEL

Since the distance between amebae is small compared to the typical distance between aggregation centers, we shall employ a continuum model. Suppose that the aggregation takes place in the  $(x, y)$  plane. For simplicity we shall assume that no quantities change with  $y$ , so that only  $x$  variation need be considered. Exercise 3 shows that this assumption of unidimensionality is not at all essential, but it makes exposition easier.

Let  $a(x, t)$  denote the number of amebae per unit area at position  $x$  and time  $t$ . Consider the amebae located in the region  $x_0 \leq x \leq x_0 + \Delta x$ , where  $\Delta x$  is an arbitrary number (not necessarily small). We shall now write a **general balance law** for this region; this states that the rate of change of the net amount of  $a$  in the region equals the rate at which  $a$  flows across the boundary, plus the net rate of creation of  $a$  within the region. In the present case,  $a$  stands for amebae, and the net creation of amebae is equal to the excess of births over deaths. But the balance law is "general" because it applies to any substance whatever. For how else can a substance appear in the region except by creation or by flow across its boundaries?

To proceed, we must define the **flux density**  $J(x_1, t)$ . This gives the net rate per unit length at which  $a$  crosses the line  $x = x_1$ . Also,  $J$  is defined to be positive if there are more amebae crossing in the direction of  $x$  increasing than in the opposite direction. The term  $Q(x, t)$  is the net rate per unit area at which  $a$  is being created. The desired balance law can now be written. Considering the rectangle of Figure 1.5, we obtain

$$\frac{\partial}{\partial t} \int_{x_0}^{x_0 + \Delta x} a(x, t) dx = J(x_0, t) - J(x_0 + \Delta x, t) + \int_{x_0}^{x_0 + \Delta x} Q(x, t) dx. \quad (1)$$

It is convenient to use the integral mean value theorem (Appendix 13.2) to write

$$\frac{\partial}{\partial t} [a(x_1, t) \Delta x] = J(x_0, t) - J(x_0 + \Delta x, t) + Q(x_2, t) \Delta x;$$

$$x_0 \leq x_1 \leq x_0 + \Delta x, \quad x_0 \leq x_2 \leq x_0 + \Delta x. \quad (2)$$

We divide by  $\Delta x$  and then take the limit as  $\Delta x \rightarrow 0$ , to obtain the **general balance law in differential equation form**,

$$\frac{\partial a}{\partial t}(x_0, t) = -\frac{\partial J}{\partial x}(x_0, t) + Q(x_0, t), \quad x_0 \text{ arbitrary.} \quad (3)$$

\* We adapt material in a paper by E. F. Keller and L. A. Segel, *J. Theoret. Biol.* 26, 399–415 (1970).

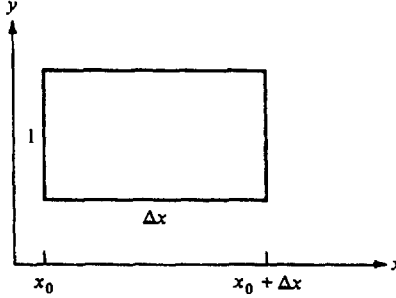


FIGURE 1.5. The balance law (3) considers the rate of change of mass in a rectangle of length  $\Delta x$  and unit width, in the case where  $y$  variation is absent.

In the case of the amebae, reproduction can be ignored because there is little or none of it in the absence of food. Deaths can also be ignored, since there are few during the time interval of interest. Thus we take

$$Q \equiv 0. \quad (4)$$

To obtain an expression for the flux  $J$ , let us first consider a situation when attractant is absent. Then the amebae appear to move about randomly. Owing to such "diffusionlike" movement, a concentration of amebae tends to disperse. Thus there is a random flux  $J_r$  from regions of high ameba concentration to regions of low concentration. The magnitude of the flux at  $x$  seems to depend on the concentration difference between  $x$  and nearby points. We characterize this difference by  $\partial a / \partial x$  (the simplest choice) and make the hypothesis that

$$J_r(x, t) = F \left[ \frac{\partial a(x, t)}{\partial x} \right] \quad (5)$$

for some function  $F$ . Now when  $a \equiv \text{constant}$ ,  $J = 0$ —for in random motion there will be as many amebae moving to the left as to the right. In this case  $\partial a / \partial x \equiv 0$ . Therefore, given (5), it is only sensible to assume that

$$F(0) = 0. \quad (6)$$

Thus the graph of  $F$  must have an appearance such as that depicted in Figure 1.6. For sufficiently small values of  $s$ , we can approximate the graph by a straight line. Calling the slope of the line  $-\mu$ , we obtain  $F(s) = -\mu s$  as the simplest reasonable assumption concerning  $F$ ; i.e.,

$$J_r(x, t) = -\mu \frac{\partial a(x, t)}{\partial x}. \quad (7)$$

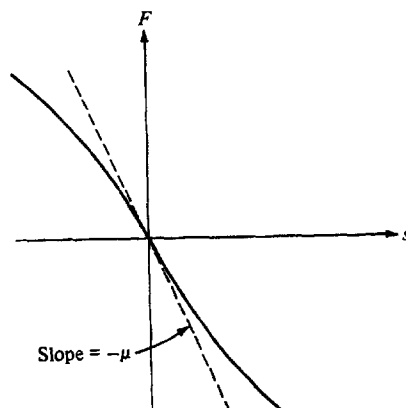


FIGURE 1.6. Possible plot of the function  $F$  defined in (5). The graph provides negative values for  $s > 0$  because there should be a leftward (negative) flux if amebae density is higher at larger values of  $x$ . Similar reasoning explains the positivity for  $s < 0$ .

Combining (3), (4), and (7), we obtain

$$\frac{\partial a}{\partial t} = \frac{\partial}{\partial x} \left( \mu \frac{\partial a}{\partial x} \right). \quad (8)$$

Equation (8) is the **diffusion equation**, which will be studied at length beginning in Chapter 3. This equation is used to model the concentration variation of any kind of randomly moving set of particles, for example smoke particles in air (see Chapter 3). The constant  $\mu$ , which governs the vigor of the random movement, is generally called the **diffusivity**. Here we call  $\mu$  the **motility**, giving precise meaning to a biological term that is often used only in a qualitative manner.

Equation (8) was obtained under the assumption that no attractant was present. To account for **chemotaxis**, which is directional motion induced by variations in chemical concentration, we add to  $J_r$  of (7) an additional contribution  $J_c$ . Let  $\rho(x, t)$  be the density of the attractant. Arguing as before, we pass from an initial assumption that  $J_c$  is a function of the attractant gradient  $\partial\rho/\partial x$  to the assumption that  $J_c$  is proportional to the attractant gradient, at least for small values of the gradient. For a given gradient, if the amebae density is twice as great, the net flux should be twice as great. The proportionality factor should thus be a multiple of  $a$ . We are led to the hypothesis that

$$J_c = \chi a \frac{\partial \rho}{\partial x}. \quad (9)$$

The factor  $\chi$  measures the strength of chemotaxis. Note that in contrast to (7), there is no negative sign in (9). This is because amebae tend to move *toward* attractant concentrations (and away from ameba concentrations).

Assuming that the total flux  $J$  in (3) is the sum of the random contribution  $J_r$  and the chemotactic contribution  $J_c$ , we are led to our final equation for the change in ameba density:

$$\boxed{\frac{\partial a}{\partial t} = \frac{\partial}{\partial x} \left( \mu \frac{\partial a}{\partial x} - \chi a \frac{\partial \rho}{\partial x} \right).} \quad (10)$$

We shall take  $\mu$  and  $\chi$  to be positive constants. It is not difficult to take into account a variation with  $\rho$  which is probably present, but this would only change some details of the analysis to come [Exercise 2(a)]. Note that even with constant  $\mu$  and  $\chi$ , (10) contains a (quadratic) nonlinear term  $\chi a(\partial \rho / \partial x)$ , because this term is proportional to the product of two unknown functions,  $a$  and  $\partial \rho / \partial x$ . [Such a quadratic nonlinearity was also found in our study of galactic structure—compare the remarks following Equation (2.4.)] Nonlinear terms generally make mathematical analysis more difficult, and hence more challenging and more rewarding.

We also need an equation for the attractant density  $\rho$ . This will be of the general form (3):

$$\frac{\partial \rho}{\partial t} = -\frac{\partial J_a}{\partial x} + Q_a.$$

(The subscript “a” refers to attractant.) The random motion of the attractant molecules will be modeled by a proportionality of flux to gradient, as in (7):

$$J_a = -D \frac{\partial \rho}{\partial x}.$$

The net creation term  $Q_a$  has a positive contribution  $fa$  as a result of the secretion of attractant by the amebae. Here  $f$  is the rate of secretion per unit ameba density. What of the decay in attractant activity? We take the rate of decay (as in radioactivity or some other spontaneous process) to be proportional to the amount of attractant present,\* via the constant  $k$ . Thus  $Q_a = fa - k\rho$ , and the desired equation for  $\partial \rho / \partial t$  is

$$\boxed{\frac{\partial \rho}{\partial t} = fa - k\rho + D \frac{\partial^2 \rho}{\partial x^2}.} \quad (11)$$

We shall take  $f$ ,  $k$ , and  $D$  to be positive constants.

\* As we stated above, decay is actually due to the action of an enzyme. If Michaelis-Menten kinetics (Chapter 10) are assumed, this can be modeled by making  $k$  a certain function of  $\rho$ . But the essentials of the analysis are unchanged [Exercise 2(b)].

Partial differential equations (10) and (11), for the two unknown functions  $a(x, t)$  and  $\rho(x, t)$ , provide the basic formulation of our problem.

#### AN EXACT SOLUTION: THE UNIFORM STATE

It is very easy to find an exact solution of (10) and (11). This is the **uniform solution**,

$$a = a_0, \quad \rho = \rho_0, \quad (12)$$

where  $a_0$  and  $\rho_0$  are constants. [When (12) holds, the system is said to be in the **uniform state**.] Upon substitution into (10) and (11), we find that (12) will indeed provide a solution if

$$fa_0 = k\rho_0. \quad (13)$$

Equation (13) is physically reasonable. It says that in the uniform state the secretion rate of attractant must be exactly balanced by the decay rate.

#### ANALYSIS OF AGGREGATION ONSET AS AN INSTABILITY

We identify the uniform state with the interphase period prior to aggregation. We also model the onset of aggregation as *the breakdown of the uniform state due to the growth of inevitable small disturbances* to ameba and attractant density. That is, we identify the onset of aggregation with the sort of **instability of the uniform state** whose investigation forms a classical part of applied mathematics (see Sections 11.1 and 15.2). The instability is presumed to occur because of changes, during interphase, of the parameters  $\mu$ ,  $\chi$ , and  $f$  that characterize ameba behavior.

The idea behind instability theory is this. Suppose that at some initial time, the state of the system is slightly disturbed from the uniform state. (Suppose that there is a local clumping of amebae, for example, and an accompanying local concentration of attractant.) Will the small disturbance tend to disappear with the passage of time or will it become more intense? In the former case we say that the uniform state is **stable to small disturbances**, in the latter **unstable**. Unstable states will not be observed, for disturbances are inevitable. In the case of instability, they will grow, so the uniform state will be replaced by some other state.

To perform a stability analysis, we introduce the variables  $a'$  and  $\rho'$  by the definitions

$$a(x, t) = a_0 + a'(x, t), \quad \rho(x, t) = \rho_0 + \rho'(x, t). \quad (14)$$

Here  $a' = a - a_0$ , for example, measures departure from uniformity; therefore, it can be identified with the disturbance in ameba density.

To obtain equations for  $a'$  and  $\rho'$ , we substitute (14) into (10) and (11). From the former we obtain

$$\frac{\partial a'}{\partial t} = \mu \frac{\partial^2 a'}{\partial x^2} - \chi \left[ (a_0 + a') \frac{\partial^2 \rho'}{\partial x^2} + \frac{\partial a'}{\partial x} \frac{\partial \rho'}{\partial x} \right]. \quad (15)$$

This equation is nonlinear, owing to the presence of the quadratic terms  $a'(\partial^2 \rho' / \partial x^2)$  and  $(\partial a' / \partial x)(\partial \rho' / \partial x)$ . We shall assume that the disturbances (and their derivatives) are small, in which case we call  $a'$  and  $\rho'$  **perturbations**. The perturbations are involved quadratically in some terms and linearly in others. Products of two small terms should be negligible in comparison with the other terms of (15), which contain but a single perturbation function. We thus **linearize** the equation by *deleting all nonlinear terms*. We obtain

$$\frac{\partial a'}{\partial t} = \mu \frac{\partial^2 a'}{\partial x^2} - \chi a_0 \frac{\partial^2 \rho'}{\partial x^2} \quad (16)$$

as the linearized version of (10). As for (11), upon substituting (14) and employing (13), we obtain

$$\frac{\partial \rho'}{\partial t} = f a' - k \rho' + D \frac{\partial^2 \rho'}{\partial x^2}, \quad (17)$$

which is already linear.

We are faced with a pair of linear partial differential equations with constant coefficients. We guess that there are solutions of the form

$$a' = C_1 \sin qx e^{\sigma t}, \quad \rho' = C_2 \sin qx e^{\sigma t}, \quad (18)$$

where  $C_1$  and  $C_2$  are constants.\* It is easily seen [Exercise 1(a)] that there are indeed solutions of the form (18), provided that

$$(\sigma + \mu q^2)C_1 - \chi a_0 q^2 C_2 = 0, \quad (19a)$$

$$-f C_1 + (\sigma + k + D q^2)C_2 = 0. \quad (19b)$$

This system of algebraic equations has the trivial solution  $C_1 = C_2 = 0$ . [From (18) we see that we have merely verified that it is possible to have an identically zero perturbation of an exact solution.] For a nontrivial solution, the determinant of the coefficients must vanish. We thus obtain the following quadratic equation for  $\sigma$ :

$$\sigma^2 + b\sigma + c = 0; \quad b \equiv k + (\mu + D)q^2, \quad c \equiv \mu q^2(k + Dq^2) - \chi a_0 f q^2. \quad (20)$$

The quadratic equation can be shown to have real roots [Exercise 1(b)]. To ensure stability, both roots must be negative, so that the exponential factor  $\exp(\sigma t)$  brings about decay of the perturbations. It is not difficult to show (Exercise 1) that a necessary and sufficient condition for stability is  $c > 0$ , which requires that

$$\chi a_0 f < \mu(k + Dq^2), \quad q \neq 0. \quad (21)$$

\* The reasons that lie behind such an assumption as (18) are more fully discussed in Section 15.2. We mention here that a cosine dependence in (18) will yield exactly the same results. (Compare Exercise 3.) Also, more general disturbances can be obtained by the superposition of sinusoidal solutions, using Fourier analysis (as discussed in Chapters 4 and 5).

From (18),  $2\pi/q$  is the wavelength of the perturbation under investigation. Since  $\mu$  and  $k$  are positive, the right side of (21) decreases monotonically as  $q$  decreases, toward a greatest lower bound of  $\mu k$ . Therefore, the longer the wavelength  $2\pi/q$ , the more “dangerous” the perturbation [for (21) is more easily violated]. We conclude that *instability* is possible whenever

$$\frac{\chi a_0 f}{\mu k} > 1, \quad (22)$$

for then (21) is violated for a range of sufficiently small values of  $q^2$ .

#### INTERPRETATION OF THE ANALYSIS

The view of aggregation that emerges is this. During the beginning of the interphase period, inequality (22) does not hold and the uniform state is stable. Triggered by the stimulus of starvation, the various parameters gradually change. Eventually, (22) is satisfied, and aggregation commences. We identify the size of the “aggregation territory” with the instability wavelength; our analysis predicts that the territory size is “very large” in some sense.

The instability criterion (22) has the following interpretation. Suppose that there is a concentration of amoebae and attractant at some point. Random “diffusion” of amoebae with motility  $\mu$  tends to disperse this concentration, as does the attractant decay, whose strength is measured by  $k$ . Larger  $\mu$  and  $k$  means larger stabilizing effects. It is therefore appropriate that increases in  $\mu$  and  $k$  mean that the instability criterion (22) is more difficult to satisfy.

By contrast, a local concentration of attractant tends to draw amoebae toward it by chemotaxis (strength  $\chi$ ). Also, a concentration of amoebae will provide a corresponding increase of attractant concentration because of the higher local concentration of secretion sources. The strength of this effect is measured by  $a_0 f$ . This explains the appearance of the destabilizing factors  $a_0 f$  and  $\chi$  in the numerator of (22). Instability ensues if destabilizing effects outweigh stabilizing.

The explanation of aggregation provided in the previous paragraph could perhaps have been provided without doing a mathematical analysis. But, as is typical, only after carrying through some calculations does one seem able to discern the physical essence of a situation. Also, the criterion (22) is a quantitative one, capable of experimental test. No full quantitative tests have been performed as yet, but it has been discovered that both the chemotactic sensitivity  $\chi$  and the attractant secretion rate  $f$  go up by about 100-fold at the time aggregation commences. As illustrated in our discussion of galactic structure, future quantitative tests might reveal defects in understanding that would never emerge from qualitative considerations.

The above analysis is based on a paper (op. cit.) that appeared in 1970 in the *Journal of Theoretical Biology*. Since that time, several other papers on slime mold aggregation, by various authors, have appeared in that journal and elsewhere. They deal with such matters as (i) a more accurate modeling of

the chemical kinetics and hence (among other things) a possible refinement of the "very large territory size" prediction mentioned above; (ii) the streaming type of aggregation, as in the frontispiece; and (iii) the influence of pulsatile attractant secretion and refractory periods. Future work will doubtless examine the effects of nonlinearity. Two survey articles on slime mold aggregation and other collective chemotactic motions, written for a mathematical audience, can be found in *Some Mathematical Questions in Biology*, Vol. III (Providence, R.I.: American Mathematical Society, 1972).

### EXERCISES

1. (a) Verify (19).  
 (b) Show that the roots of the quadratic equation (20) are real.  
 (c) Show that both roots are negative if and only if both  $b$  and  $c$  are positive.  
 (d) Show that since  $f$ ,  $D$ , and  $\chi$  are positive, if  $c > 0$ , then  $b > 0$ . Thereby deduce (21).
2. Investigate the changes in the analysis that would occur under the following conditions.  
 (a) If  $\mu$  and  $\chi$  were functions of  $\rho$ .  
 (b) If  $k$  and  $f$  were functions of  $\rho$ .
3. If two-dimensional variation is considered, the governing equations (10) and (11) are replaced by

$$\frac{\partial a}{\partial t} = \nabla \cdot (\mu \nabla a - \chi a \nabla \rho), \quad \frac{\partial \rho}{\partial t} = f a - k \rho + D \nabla^2 \rho.$$

Assume disturbances with spatial dependence  $\sin(q_1 x + q_2 y + \theta)$ , where  $q_1$ ,  $q_2$ , and  $\theta$  are constants. Show that if  $q^2 = q_1^2 + q_2^2$ , then  $2\pi/q$  remains the disturbance period and the instability condition remains (22).

4. Define  $\Delta$  by  $\chi a_0 f / \mu k = 1 + \Delta$  and suppose that  $\Delta$  is small and positive. [Compare (22).] Ignoring higher order terms in  $\Delta$ , find an approximation for the larger root of the quadratic. Deduce that the wavelength of the fastest growing disturbance is approximately  $2\pi(2D/k\Delta)^{1/2}$ .

## Appendix 1.1 Some Views on Applied Mathematics

### ON THE NATURE OF APPLIED MATHEMATICS

I . . . describe applied mathematics as the bridge connecting pure mathematics with science and technology. I have deliberately described this bridge as *connecting* two areas of activity rather than *leading* from one to the other, because the bridge carries two-way traffic. Its importance to science and technology is obvious, but it is not less important to pure mathematics, which would be poorer without the stimuli coming from the applications.

W. Prager, "Introductory Remarks" in the special issue, "Symposium on the Future of Applied Mathematics," *Quart. Appl. Math.* 30, 1-9 (1972).