COMPUTER SIMULATION OF RADIAL IMMUNODIFFUSION

I. Selection of an Algorithm for the Diffusion Process

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ABSTRACT Theories of diffusion with chemical reaction are reviewed as to their contributions toward developing an algorithm needed for computer simulation of immunodiffusion. The Spiers-Augustin moving sink and the Engelberg stationary sink theories show how the antibody-antigen reaction can be incorporated into boundary conditions of the free diffusion differential equations. For this, a stoichiometric precipitate was assumed and the location of precipitin lines could be predicted. The Hill simultaneous linear adsorption theory provides a mathematical device for including another special type of antibody-antigen reaction in antigen excess regions of the gel. It permits an explanation for the lowered antigen diffusion coefficient, observed in the Oudin arrangement of single linear diffusion, but does not enable prediction of the location of precipitin lines. The most promising mathematical approach for a general solution is implied in the Augustin alternating cycle theory. This assumes the immunodiffusion process can be evaluated by alternating computation cycles: free diffusion without chemical reaction and chemical reaction without diffusion. The algorithm for the free diffusion update cycle, extended to both linear and radial geometries, is given in detail since it was based on gross flow rather than more conventional expressions in terms of net flow. Limitations on the numerical integration process using this algorithm are illustrated for free diffusion from a cylindrical well.

INTRODUCTION

The discovery that the precipitin reaction between antibody and antigen molecules could occur in transparent gels led to the exceedingly practical field of immunodiffusion (Crowle, 1961). The enormous variety of experimental arrangements, employing one- or two-dimensional configurations with or without separation of the antibody and antigen reservoirs, all conceptually involve "simultaneous diffusion and chemical reaction." The differential equation of the process is (Crank, 1956, p. 5, 121).

$$\partial c/\partial t = \text{div } (D \text{ grad } c) - \partial b/\partial t,$$

where c is the concentration of reactant (say, antibody or antigen) in its free state, b is its bound concentration, and D is the diffusion coefficient, which may be a function of the space coordinates, the free concentration, the bound concentration, and the time t. Milne (1970, p. 4) points out the "horrible sense of frustration" when one realizes that he has neatly formulated his problem in differential equations, like the above equation, but gets no further because none of the clever devices used to achieve an analytical solution apply. An alternative is to use numerical methods.

One form of numerical analysis involves computer simulation. As defined by Martin (1968), a *computer simulation model* is a logical mathematical representation of a concept, system, or operation programmed for solution on a high speed electronic computer. Martin proposed that computer simulation should be applied whenever (*a*) analytical tools are unavailable or inappropriate, (*b*) there is reasonable assurance that sufficient data and information are obtainable to give realism to the model, (*c*) a large volume of computations is necessary, and (*d*) the mere process of constructing the model can be a beneficial learning experience concerning the concept, system, or operation simulated (paraphrased from p. 15, Martin, 1968). I feel that all four of these reasons apply to immunodiffusion and hope to show in this series of papers how computer simulation concepts can be used to solve the above differential equation. In particular, the model consists of alternating computation cycles of diffusion without reaction and reaction without diffusion.

The sequence of computational steps that gives the solution to all problems of a specified type is called an *algorithm*. There will be two major categories of algorithms required: one representing the first term on the right of the above equation for the diffusion and one representing the second term for the chemical reaction. This paper starts with a review of some of the attempts at an analytical solution and shows the first two of Martin's criteria are satisfied. This part ends with the algorithm chosen for free diffusion and an illustration of the large number of computations required in accordance with the third criterion. Part II gives the selection process used to arrive at an algorithm for the chemical reaction, and part III gives the results of the simulation, which, hopefully, satisfy Martin's fourth criterion, and explain the quantitative relationships found for radial immunodiffusion (Trautman et al., 1971).

NOTATION

The various experimental arrangements for immunodiffusion are well known. Here, in Figs. 1 A–F, they are classified as either one- or two-dimensional and as either single or double diffusion. Both reagents must have a reservoir, whether it be in a well or by impregnation of the gel matrix; hence, "single" actually means there is no initial separation of the reservoirs and "double" means there is. Both reagents may diffuse no matter what geometrical arrangements are employed. For systems

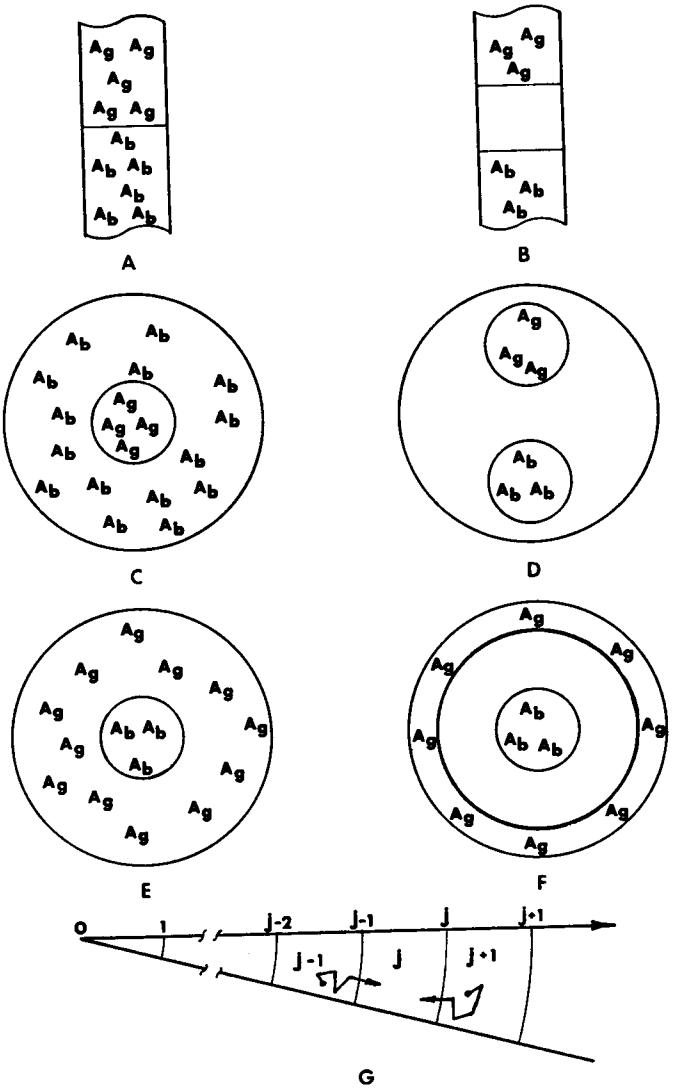

FIGURE 1 Immunodiffusion arrangements. Initial loading with antibody (Ab) and antigen (Ag) is shown. One-dimensional: A, B. (A) Forward, single if Ag is in excess; reverse, single if Ab is in excess at initial boundary (Oudin, 1952). (B) Double (Oakley and Fulthorpe, 1953). Two-dimensional: C, D, E, F. (C) Forward, single, radial (Mancini et al., 1965). (D) Double (Ouchterlony, 1948). (E) Reverse, single, radial (Vaerman et al., 1969). (F) Concentric, double (Aladjem et al., 1968). Notation for analysis of radial arrangements (C, E, F). (G) Gel of thickness h is divided into annular compartments of width Δl and numbered consecutively. For compartment j, concentration is c_j, volume is $\pi(2j - 1)h(\Delta l)^2$, and flow from neighboring compartments is F_j through outer border of cross-sectional area $2\pi jh\Delta l$ and F_{j-1} through inner border of area $2\pi(j - 1)h\Delta l$.

where one of the reagents may be too large to diffuse only the single method can be employed. Fig. 1 G gives the notation for the simulation model in which the gel is divided into computational compartments.

SPIERS-AUGUSTIN MOVING SINK THEORY

Derivation

A successful data processing theory of forward linear single diffusion of the Oudin (1952) arrangement (Fig. 1 A) was given by Spiers and Augustin (1958). The basic concept was to incorporate the antibody-antigen reaction into the mathematical boundary conditions of the free diffusion differential equations (Adair, 1920; Crank, 1956, p. 111).

Letting l be the one-dimensional coordinate measured downwards from the initial boundary in Fig. 1 A, Fick's first and second laws are written from left to right, respectively, for each reagent diffusing separately as

$$\begin{aligned} F_{Ag} &= -D_{Ag}\partial c_{Ag}/\partial l, \qquad \partial c_{Ag}/\partial t = D_{Ag}\partial^2 c_{Ag}/\partial l^2, \qquad \text{for} \quad l \leq L, \\ F_{Ab} &= -D_{Ab}\partial c_{Ab}/\partial l, \qquad \partial c_{Ab}/\partial t = D_{Ab}\partial^2 c_{Ab}/\partial l^2, \qquad \text{for} \quad l \geq L, \end{aligned} \tag{1}$$

where F_i is the flow per unit area, D_i the diffusion coefficient, and c_i the concentration. Fick's second law is seen to be the equation in the introduction without the reaction term. The moving boundary condition, called here a moving "sink" (Augustin, 1957; Aladjem et al., 1962), assumes (*a*) each free reagent is removed from the system at the moving level L of the precipitate line, and (*b*) the attempt for either reagent to cross is met by its precipitation in a compound of definite weight proportions R. These two separate conditions couple the diffusion of the two reagents such that

$$c_{Ab} = c_{Ag} = 0, \qquad RF_{Ag} + F_{Ab} = 0, \qquad \text{at} \quad l = L. \tag{2}$$

The solution of the second order "parabolic" differential equation 1 subject to equations 2 was given as

$$R\left(\frac{c^0_{Ag}}{c^0_{Ab}}\right)\left(\frac{D_{Ag}}{D_{Ab}}\right)^{1/2} = \frac{\{1 + \operatorname{erf}[s/(4D_{Ag})^{1/2}]\}\exp[s^2/(4D_{Ag})]}{\{1 - \operatorname{erf}[s/(4D_{Ab})^{1/2}]\}\exp[s^2/(4D_{Ab})]}, \tag{3}$$

in which erf is the error function and exp is the exponential function and

$$L = s(t)^{1/2}. \tag{4}$$

Equation 3 shows that the slope s is implicitly related to initial concentrations, denoted by superscript zero, diffusion coefficients, and the composition of the precipitate. Hence, s is constant in equation 4, which applies to each Oudin tube. The

initial concentrations for which there will be no movement of the precipitate front can be found by setting $s = 0$. Since erf(0) and exp(0) are both unity, equation 3 yields

$$c^0_{Ab}/c^0_{Ag} = R(D_{Ag}/D_{Ab})^{1/2}. \tag{5}$$

The solution of equations 1 and 2 also yields the "density," in concentration units, of antibody and antigen in the precipitate as

$$b_{Ab} = \left(\frac{2(D_{Ab})^{1/2}\exp[-s^2/(4D_{Ab})]}{s(\pi)^{1/2}\{1 - \text{erf}[s/(4D_{Ab})^{1/2}]\}}\right) c^0_{Ab}, \qquad b_{Ag} = b_{Ab}/R. \tag{6}$$

The limit of b_{Ab} for a fast moving front ($s \to \infty$) is c^0_{Ab}, but for a stationary band ($s \to 0$) it is ∞. Taking equations 5 and 6 together, it is seen that the precipitate has a constant ratio R of antibody to antigen that is independent of time or quantities of reagents used, but the ratio of initial concentrations required to prevent movement of its leading edge is R times a factor depending on the diffusion coefficients. This is a fundamental concept, seemingly missed even in recent reviews (Polson, 1971). At this point, R is not required to be the equivalence ratio.

Becker and Neff (1958) independently published the equivalent of formulas 3 and 4 based on the same concepts. Since Spiers and Augustin provided the most general case, they are credited with the theory. Their general case replaces the 1 + erf term in the numerator of equation 3 with $(D_{Ag}/D'_{Ag})^{1/2}$ + erf, where D'_{Ag} is the diffusion coefficient of the antigen in its reservoir, which may be different from its coefficient when in the Ab reservoir where the reaction takes place. If the Ag reservoir contains no agar and is stirred, $D'_{Ag} = \infty$; but, if agar is used and the antigen is diluted in normal serum to the same viscosity as the immune system used, then $D'_{Ag} = D_{Ag}$, the case considered in equation 3. If there is a viscosity difference between the reservoirs, presumably $D_{Ag}/D'_{Ag} = \eta_{Ag}/\eta_{Ab}$ where η_i refers to the viscosity in the respective reservoirs.

Verification and Discovery of Limit on Antiserum Concentration

The Spiers-Augustin theory has three adjustable parameters: D_{Ag}, D_{Ab}, and R. The verification consists of showing that the forms of equations 3 and 4 are met by the data, and then that the values of the three parameters bear some resemblance to their free solution counterparts.

Spiers and Augustin (1958) proposed a graphical method for determining all three parameters, and Augustin et al. (1958) tried it out. The experiment consisted of measuring the displacement of the precipitate front at several times in a series of Oudin tubes at different initial concentrations of antigen. The detailed graphs of L vs. $(t)^{1/2}$ were published and do appear linear to the eye. Thus, the form of equation 4 is verified. Then, theoretical curves, computed from the nonlinear equation 3, were constructed for various values of the parameters. By a translation process on a

log scale, the theoretical curve closest to the experimental points was selected. For the bovine serum albumin (BSA)-rabbit immunoglobulin G (IgG) system at 20°C in 0.5% agar and ~3% serum, these parameters were $D_{Ag} = 5.3$ F, $D_{Ab} = 4.7$ F, and $R = 4.7$. The graphical method did not permit internal standard errors to be computed and so the comparison with free solution values is without confidence.

Becker (1961) realized that precision goes up as the number of parameters to be determined goes down. He also recognized that if he could "linearize" equation 3, he could use least squares curve-fitting procedures to obtain both a slope and an intercept with their internal standard errors. His proposal for unweighted linear least squares will be termed here the Becker two-parameter method, and amounts to writing equation 3 as

$$\ln\left[\left(\frac{c^0_{Ag}}{c^0_{Ab}}\right)\left(\frac{1 - \operatorname{erf}[s/(4D_2)^{1/2}]}{1 + \operatorname{erf}[s/4(D_1)^{1/2}]}\right)\right] = -\left(1 - \frac{D_{Ab}}{D_{Ag}}\right)\left(\frac{s^2}{4D_2}\right) + \ln\left[\frac{1}{R}\left(\frac{D_{Ab}}{D_{Ag}}\right)^{1/2}\right], \quad (7)$$

where D_1 and D_2 are provisional (trial) values of D_{Ag} and D_{Ab}, respectively. If the provisional values are close to the true values, then by plotting the left-hand side against $s^2/(4D_2)$ a straight line should result that does not require a provisional value of the third parameter R. The slope $\tilde{b}$ can then be converted to D_{Ag} and its standard error with

$$\begin{aligned} \tilde{b} &\equiv -(1 - D_{Ab}/D_{Ag}), \\ D_{Ag} &= D_{Ab}/(1 + \tilde{b}), \\ \sigma_{D_{Ag}} &= D_{Ag}\sigma_{\tilde{b}}/(1 + \tilde{b}) \end{aligned} \quad (8)$$

where $\sigma_{\tilde{b}}$ is the standard error of the slope and D_{Ab} is taken as D_2. Becker found that the value of D_{Ag} was relatively insensitive to both trial values but that the linearity depended on D_2. Hence, with just one or two trials, D_1 and D_2 can be selected so that the plot is linear with its slope returning the same value D_1 for D_{Ag}. Then, the intercept $\tilde{a}$ can be converted to R by

$$\begin{aligned} a &\equiv \ln\left[\frac{1}{R}\left(\frac{D_{Ab}}{D_{Ag}}\right)^{1/2}\right], \\ R &= (1 + \tilde{b})^{1/2} \exp(-\tilde{a}), \\ \sigma_R &= R\left[\sigma^2\tilde{a} + \frac{1}{4(1 + \tilde{b})}\left(\frac{1}{1 + \tilde{b}} + \bar{S}\right)\right]^{1/2}, \end{aligned} \quad (9)$$

where $\bar{S}$ in the standard error formula is the mean of the $s^2/(4D_2)$ values used in the least squares curve fitting and provides the covariance contribution because $\tilde{a}$ and $\tilde{b}$ are not independently determined. The standard error formulas of equations 8 and

TABLE I

VERIFICATION OF SPIERS-AUGUSTIN THEORY AT LOW ANTIBODY CONCENTRATIONS

Two-parameter least squares method*							
Serum		No. of points‡	Trial§ D_1	Curvature‖	Composition¶ R	Diffusion coefficient D_{Ag}	
						Observed**	Corrected‡‡
% v/v	mg N/ml				w/w		
1.5	0.0137	12	6.01	1.16	5.8 ±0.2	6.00 ±0.06	6.22 ±0.06
3.0	0.0274	10	5.96	0.31	5.5 ±0.2	5.96 ±0.07	6.22 ±0.07
					5.7 ±0.2		6.22 ±0.04
		Expected values		<2.3‖	7.46 − 5.85§§		6.87 ±0.01‖‖

Linear, forward, single diffusion for BSA-rabbit IgG system, 25.00°C ±0.05, 0.3% agar (Becker and Neff, 1958)

* D_{Ab} assumed, D_{Ag} and R computed from slope and intercept of special semilog plot for various initial concentrations of antigen (Becker, 1961).

‡ Antigen diluted from 14.81 mg N/ml in approximately twofold steps.

§ Diffusion coefficients (Fick units) for antigen on second iteration. D_2 for antibody assumed as 3.9 F.

‖ Curvature tested with quadratic least squares; value listed is Student's t for second-degree coefficient. For 95% confidence of curvature, value must be >2.262 and >2.365, respectively.

¶ Computed ratio of Ab to Ag in precipitate from slope and intercept, given with ±1 SE.

** Computed from slope and trial value of D_2, given with ±1 SE.

‡‡ Corrected for viscosity of serum, assumed to be 1.036 and 1.043, respectively.

§§ Precipitin analysis; range is equivalence zone from Ab to Ag side, as given by authors.

‖‖ Converted from 1° to 25°C using $D_{25} = [T_{25\eta_1}/(T_{1\eta_{25}})]D_1$ and $D_1 = 3.261 \pm 0.004$ F at pH 5.1 in 0.5 M KCl (Wagner and Scheraga, 1956) and viscosities for water.

9 were not given by Becker (1961, 1971) but were deduced by application of the transmission of variance formula $\sigma_z^2 = (\partial f/\partial x)^2\sigma_x^2 + (\partial f/\partial y)^2\sigma_y^2 + 2(\partial f/\partial x)(\partial f/\partial y)$ cov (x, y) where $z = f(x, y)$ and cov is the covariance of x and y (Deming, 1964). It must be noted that unweighted least squares were used, even though the transformation required in equation 7 is nonlinear.

The most complete data for checking this theory have been given by Becker and Neff (1958). Four antiserum concentrations were used, each with 10–12 dilutions of antigen. The positions of the precipitate lines were measured and found to be linearly related to $(t)^{1/2}$. Neither these data nor their plots were given, but the appropriate slopes were. Becker (1961) used the highest dilution of antiserum (1.5%) as a test and presented a graph that showed his proposed log plot, according to equation 7, was linear. The numerical results for 1.5 and 3.0% antiserum have been recomputed and are given in Table I.[1] These are from the second iterative cycle with the

[1] The programs used for the Olivetti Programma 101 Desk-Top computer (Olivetti Underwood Corp., New York) are available from the author. Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

TABLE II

FAILURE OF SPIERS-AUGUSTIN THEORY AT HIGH ANTIBODY CONCENTRATION

Two-parameter least squares method*					
No. of points‡	Trial§ D_2	Curvature‖	R¶	D_{Ag}**	Viscosity ratio assumed‡‡
8	3.9	3.1	5.37 ±0.39	4.92 ±0.15	1
8	3.5	3.1	5.22 ±0.37	4.95 ±0.15	1
8	3.0	3.0	5.03 ±0.34	4.99 ±0.15	1
8	2.5	2.9	4.82 ±0.31	5.03 ±0.14	1
8	2.0	1.8	4.67 ±0.28	5.03 ±0.13	1
6§§	3.0	0.9	4.19 ±0.22	5.28 ±0.11	1
8	3.0	4.2	4.72 ±0.36	5.13 ±0.17	variable
6§§	3.0	2.0	3.84 ±0.23	5.48 ±0.14	variable
8	3.0	0.1	1.83 ±0.04	5.48 ±0.06	0
Expected values		<2.6‖‖	5.7 ±0.2¶¶	5.54 ±0.04***	

Linear, forward, single diffusion for BSA-rabbit IgG system, 25% (v/v) antiserum, c^0_{Ab} = 0.228 mg N/ml, variable antigen, 25.00°C ±0.05, 0.3% agar (Becker and Neff, 1958).

*, ‡, §, ‖, ¶, ** See footnotes *, ‡, §, ‖, ¶, ** , Table I; the raw data used are given in Table III.

‡‡ $(D_{Ag}/D'_{Ag})^{1/2}$ term used to allow for variation between diffusion of antigen in antigen reservoir (D_{Ag}) and in antibody reservoir (D_{Ag}). Its value is: 1, no correction; 0, stirred antigen solution; $(\eta_{Ag}/\eta_{Ab})^{1/2}$ for cases called "variable" (see Table III for viscosity data assumed).

§§ Omitting the two lowest antigen concentration points (see Table III).

‖‖ Student's t for 5 degrees of freedom, 95% confidence level.

¶¶ From Table I, composition average.

*** From Table I, D corrected for assumed viscosity: 6.22/1.123 = 5.54.

trial values listed The "curvature" is given in the fifth column in terms of the quadratic term divided by its standard error (Student's t value) when parabolic least squares are used. Because the values are less than Student's t for 9 and 7 degrees of freedom for the 1.5 and 3.0% antiserum, respectively, the function is not curved. The value for D_{Ag} (column 7) is the same as the trial value (column 4). The final results for the two parameters, R and D_{Ag}, in the two sets of data are not significantly different from their trial values, in accordance with the theory. The values of the parameters, however, must further be compared with free solution values. The computed composition ratio in the precipitate, R = 5.7 ±0.2, is on the antigen excess side of the equivalence zone that extended from a weight ratio of 7.46 down to 5.85. In the case of D_{Ag}, 6.22 ±0.04 is significantly lower than the free solution value of 6.87 ±0.01 F converted from the data at 1°C of Wagner and Scheraga (1956) or the range of values for 25°C, 6.66–6.81 F, given by Gosting (1956).

The same analysis for the highest concentration of serum (25%) is given separately, since it reveals a limitation on the applicability of the theory. In Table II the results for a series of trial values are given. The first five rows are comparable with either row in Table I using all the data and ignoring any difference in the diffusion coefficient of the antigen in the two reservoirs. The Student's t value for the curvature

TABLE III

COMPOSITION AND DENSITY OF PRECIPITATE COMPUTED FROM SPIERS-AUGUSTIN THEORY AT HIGH ANTIBODY CONCENTRATION

Data			Computation*	
c^0_{Ag}	s	η_{Ag}‡	R	$b_{Ab} + b_{Ag}$
mg N/ml	*10^{-13} cm/sec$^{1/2}$*	*centipoise*	*w/w*	*mg N/ml*
14.81	2.767	1.625	4.09	0.300
7.259	2.542	1.260	4.40	0.298
3.557	2.223	1.128	3.87	0.310
1.743	1.947	1.074	4.06	0.313
0.854	1.638	1.051	4.17	0.322
0.418	1.302	1.040	4.30	0.337
0.205	0.982	1.035	4.78	0.361
0.100	0.678	1.032	5.67	0.412
		Expected values	6.66§	0.265‖ for $s = \infty$ ∞ for $s = 0$

Same experiment as for Table II.

* $D_{Ab} = 3.0\,\mathfrak{F}$, $D_{Ag} = 5.3\,\mathfrak{F}$, no correction for viscosity (same as sixth row of Table II, see text for equation.

‡ Not given by authors, computed from $(\eta/\eta_w - 1)/c^0_{Ag} = 0.0365 + 0.0028\ c^0_{Ag}$ (Tanford and Buzzell, 1956, for pH 7.3, μ 0.1), using $\eta_w = 1.030$ centipoise as the buffer; $\eta_{Ab} = 1.123$ centi poise assumed for 25% (v/v) serum.

§ Average of extremes of precipitin zone values given in Table I (7.46 and 5.85).

‖ Computed from $c^0_{Ab}\ (1 + 1/R)$; $c^0_{Ab} = 0.228$, $R = 6.66$.

test is given in column 3. As suggested by Becker (1961), the curvature does become less as the trial value of D_2 is reduced; however, as he observed, the very low value of 2.0 $\mathfrak{F}$ to achieve linearity cannot correspond to reality.

The four rows in the lower part of Table II show the results of other manipulations that might be tried to make the theory fit. It will be necessary to also refer to Table III, where the raw data are given. First, notice that the viscosity of the 25% serum is, perhaps, 1.123 and the trial value of D_2 could at least be reduced to 3.9/1.123 = 3.4. Instead, for the series of computations listed in the lower part of Table II, a compromise value of $D_2 = 3.0\ \mathfrak{F}$ was chosen. Second, observe for row 6 that by discarding the lowest two antigen concentrations, the curvature is removed. Third, consider the enormous difference in viscosity that probably exists between the two reservoir layers (third column of Table III). The formula for making the correction due to this effect has been provided by Spiers and Augustin (see above), and the results are given in rows 7 and 8 of Table II. It is seen that this correction makes the curvature worse when using all the points as well as for the selected six points. Fourth, notice that if the antigen reservoir is fictitiously assumed to be completely stirred ($D'_{Ag} = \infty$), the last row of calculations show that the curvature can be eliminated. In so doing, the antigen diffusion coefficient becomes reasonable (5.48 ±

0.06 compared with the "expected" value of 5.54 $\pm$0.04), but the composition ratio $R = 1.83 \pm 0.04$ is not realistic.

These difficulties are not unique to this one set of data, for the 6% serum results, not displayed in detail here, already show such a trend. Also, the Augustin et al. (1958) data for ~3% serum give curvature and underestimated values for the parameters when processed in the manner of Table I. For reference, these values (at 20°C $\pm$1) are that $D_1 = 5.4$ and $D_2 = 3.0$ F yield $D_{Ag} = 5.40 \pm 0.05$ F, $R = 4.3 \pm 0.1$, and curvature = 2.9 for 8 degrees of freedom. Free solution values were cited as 6.1 F and 6.6, respectively, for D_{Ag} and R.

Reason for Discrepancy from Optical Scanning of Gels

The density of the precipitate can be computed from the Spiers-Augustin theory using the final values found for the parameters and equation 6. As an illustration, values are given in column 5 of Table III for reasonable values of the parameters. These densities show that as the antigen concentration is reduced and as the converted speed s of the moving front of the precipitate goes down, the density goes up. This is completely in accord with the optical scanning results and interpretation of Hayden and Becker (1960) on a similar system (ovalbumin-rabbit IgG) at a given time. The scans, however, while verifying the predicted density at the leading edge, show the precipitate to be in the form of a line, having been solubilized behind its moving front (see Oudin, 1952; Glenn, 1956, for examples). Furthermore, the leading edge was found to increase its density with time, especially at high concentrations of antibody. Hence, one reason given by all the authors why the Spiers-Augustin theory does not always fit is that the antibody-antigen reaction is more complicated than merely a moving sink. The reason it does fit at high antigen and low antibody concentrations, but with a lowered diffusion coefficient, follows from Hill's theory below.

Unsuitability as an Algorithm

The Spiers-Augustin theory is not suitable as an algorithm for simulation purposes because it is not sufficiently general and because it uses an "effect" (moving boundary condition) rather than a "mechanism." It provides, however, a rigorous analytical solution for a model system in which a stoichiometric precipitate is laid down and neither added to nor solubilized behind its leading edge. Its limited success does show that the precipitate is *not* a physical barrier for antigen, and a moving front does *not* theoretically require resolubilization. The reverse Oudin method has been tried (Oudin, 1952; Augustin, 1957) and showed that even antibody can pass through a precipitate and that, experimentally, resolubilization is not required for movement. Unfortunately, the data have not been published and have not been analyzed critically to see if the composition of the precipitate is on the antibody excess side of equivalence.

ENGELBERG STATIONARY SINK THEORY

Derivation

Engelberg (1959) seems to have been the first to use immobility of the precipitin line in linear double diffusion (Fig. 1 B) as a mathematical device to solve the diffusion equations. This improved the "free diffusion with indicator" theory in which the influence of the reaction was ignored (Polson, 1958, 1971).

Polson's "quantitative gel precipitin" technique for the Oakley and Fulthorpe arrangement involves a special apparatus for precise layering of the three agar columns: the lowest contains the antibody, the highest the antigen, and the center column is initially free of both reagents (Fig. 1 B). A series of such columns is set up with a constant concentration of antiserum, but with a dilution set of antigen extending on both sides of equivalence. A clear set of data is given graphically by Regenmortel (1959); there seems to be no tabular data published. The interesting experimental fact is that conditions giving minimum band width and nonmovement of the band coincide. At this point, the system is said to be "balanced." The band width measurements are used to locate this condition, not for calculations of any fundamental parameters.

Consider only the balanced column and assume that above the precipitate there is unrestricted diffusion of the antigen: no antibody is present, but at the line its free concentration is maintained at zero because of precipitation. The mathematical solution can be written using the solution of the right-hand equation 1 for unrestricted diffusion from an unstirred reservoir at $l = 0$

$$c/c^0 = (\tfrac{1}{2})\{1 - \operatorname{erf}[(l/(4Dt)^{1/2}]\}, \tag{10}$$

and the mathematical device of negative reflection (Crank, 1956, p. 15) at the boundary of the sink $l = L$

$$2c/c^0 = \{1 - \operatorname{erf}(l/(4Dt)^{1/2}\} - \{1 - \operatorname{erf}[(2L - l)/(4Dt)^{1/2}]\}. \tag{11}$$

From the left-hand equation 1, the flow into the sink is

$$F = -D(\partial c/\partial l)_{l=L} = c^0[D/(4\pi t)]^{1/2} \exp\,[-L^2/(4Dt)], \tag{12}$$

which is exactly double the amount at that level if there had been no sink. The computations of Aladjem (1964) show this factor of two even in the two-dimensional case where the corresponding equation involves Bessel functions (cited as 1.9).

Separate equations can be written for both the antigen and the antibody, choosing the common sink for both to be located at the distance L_{Ag} from the antigen reservoir and L_{Ab} from the antibody reservoir in Fig. 1 B. The absolute value of the ratio of *flows* there, and only there, can be written from equation 12 as

$$\left|\frac{F_{Ab}}{F_{Ag}}\right| = \left(\frac{c^0_{Ab}}{c^0_{Ag}}\right)\left(\frac{D_{Ab}}{D_{Ag}}\right)^{1/2} \exp\left[-\frac{1}{4t}\left(\frac{L^2_{Ab}}{D_{Ab}} - \frac{L^2_{Ag}}{D_{Ag}}\right)\right] \equiv R, \tag{13}$$

where R, at this point in the derivation, is defined by equation 13. It is now necessary to deduce the properties of R. First, in a small increment of time, R is also the ratio of the *amounts* of reagents that cross and precipitate. Hence, it is the *composition* ratio of the precipitate. Second, note that for R to be independent of time, the argument of the exponential function must vanish. Fortunately, this gives Polson's formula for diffusion coefficients

$$D_{Ag}/D_{Ab} = (L_{Ag}/L_{Ab})^2. \tag{14}$$

This simultaneously requires, however, from equation 13, that

$$R = (c^0_{Ab}/c^0_{Ag})(D_{Ab}/D_{Ag})^{1/2} = (c^0_{Ab}/c^0_{Ag})(L_{Ab}/L_{Ag}), \tag{15}$$

which differs from Polson's result for balance, but is seen to be the same as that of the Spiers-Augustin theory for the Oudin method, equation 5, as pointed out by Engelberg (1959). This is not surprising because the same differential equations 1 were used for both geometrical arrangements and a stationary line is a special case of the moving boundary condition of equation 2.

Verification

No data have been published on the conditions for balance or on the precipitate composition as a function of time. Instead, interest has centered on measuring diffusion coefficients of antigens.

The modification by Allison and Humphrey (1960) using two troughs at right angles provides, in effect, multiple readings for the same system. Their tabulation shows excellent agreement of measured diffusion coefficients, using the equivalent of equation 14, with free solution values. A recent summary by Polson (1971) also shows general agreement with other methods, however, no standard errors were given and confidence levels cannot be assigned. It is important to note that Polson used horse IgG as the reference; not only does it solubilize in antibody excess, but in his laboratory it has a significantly higher diffusion coefficient than does rabbit IgG.

Allison and Humphrey (1960) presented the distributions of antibody and antigen in two-dimensional double diffusion under several conditions. They suggested that soluble complex formation as well as imbalance had an effect on the movement of the precipitin line. Aladjem (1964) also found that the stationary sink theory was too restrictive a model for double diffusion from isolated wells.

Unsuitability as an Algorithm

The stationary sink theory is not useful as the basis of an algorithm for simulation, because it, like the moving sink theory, contains the very special "immune barrier" condition in order to achieve data processing formulas. Furthermore, this theory applies only to the balanced state. Both theories do show that diffusion can be sepa-

rated from the chemical reaction in the special case where the reaction results in a stoichiometric precipitate at a boundary. They also indicate that for a precipitate in immunodiffusion to have a certain composition R whether it appears to move or not, the ratio of *flows* must have that ratio, not the ratio of concentrations. This is in sharp contrast to the conditions for equivalence in precipitin analysis.

HILL SIMULTANEOUS LINEAR ADSORPTION THEORY

Derivation

Hill (1968) considered the reaction to be everywhere simultaneous with diffusion. The case treated was forward single radial immunodiffusion, Fig. 1 C, but only for the region inside the precipitate ring itself. He used the equation in the introduction, expressed in cylindrical coordinates as (Crank, 1956, p. 5, 122)

$$\frac{\partial c}{\partial t} = \frac{1}{l}\left[\frac{\partial}{\partial l}\left(lD\,\frac{\partial c}{\partial l}\right)\right] - \frac{\partial c_b}{\partial t}, \tag{16}$$

where l is taken as the radius to avoid introducing another symbol, and c_b is the "concentration" of immobilized (bound) antigen. Hill assumed a "linear adsorption isotherm," i.e., that

$$c_b = kc. \tag{17}$$

With this, equation 16 can be written as

$$\frac{\partial c}{\partial t} = \frac{1}{l}\left\{\frac{\partial}{\partial l}\left[l\left(\frac{D}{k+1}\right)\frac{\partial c}{\partial l}\right]\right\}. \tag{18}$$

The interpretation given to equation 18 is that the functional relationship for diffusion with a linear adsorption reaction is the same as without it, but that the free diffusion coefficient is reduced to $D/(k+1)$.

Verification

Unfortunately, Hill's attempt to verify his theory was abortive. He measured the *total* antigen rather than the free antigen and he ignored the mechanism that lead to the precipitation ring. His concept, however, can be applied to the simpler linear case of Fig. 1 A where the Spiers-Augustin theory accounted for the moving edge of the precipitate but failed to predict a reduced diffusion coefficient. If the reaction of the antigen with the precipitate *behind* the front, which solubilizes it, is linear, Hill's theory would explain both why the free diffusion equations seem to work and why the observed diffusion coefficient is too small. The need for two reactions, one for the the resolubilization and one for the precipitation, shows that the eventual simulation of radial immunodiffusion will have to consider much more complicated mechanisms than any of these theories reviewed here have.

AUGUSTIN ALTERNATING CYCLE THEORY

Concept

Augustin (1957) seems to be the first to have simulated immunodiffusion. She divided an Oudin type tube into compartments and alternated calculation cycles of free diffusion without reaction and then instantaneous antibody-antigen reaction without diffusion. One hand-computed trial for 7 compartments that were updated for 11 time periods was given for a balanced system. The algorithm for the antibody-antigen reaction that was applied in each compartment specified instantaneous precipitation in equivalence proportions of the reagent in shorter supply. For the diffusion algorithm, she let one-fourth of the amount in each compartment flow in each time period to each of the compartments on either side.

This alternating cycle method of handling simultaneous diffusion and chemical reaction appears the most general since any initial conditions can be selected and various reaction mechanisms, no matter how complicated, can be tried. Incidentally, this method was again proposed, but not tried, by Aladjem (1964). It assumes (*a*) that the "coupling" between the two processes can be handled by letting the output of one be the input of the other, and (*b*) that the numerical solution will converge to the analytical solution if the "mesh" size (to be explained) is chosen sufficiently small. In principle, these assumptions can be checked in any given trial by (*a*) reducing the mesh size and (*b*) by interchanging the order of the update calculations. If the solutions are "not too far apart," either one can be considered the answer.

Inclusion of a force field along with diffusion and interaction has been considered for the centrifuge in a series by Cox (1965). For an extensive review of this and other approaches in electrophoresis and chromatography see the book by Cann (1970), and for a similar approach in epidemiology see the book by Watt (1968). The Augustin theory forms the basis of the computer simulation of this series, and her diffusion algorithm will now be given in detail as extended for radial geometry.

Algorithm for Radial Diffusion

The algorithm is based on division of the gel into computational compartments (Fig. 1 G) into each of which hypothetical "partial" flows from both of its immediate neighbors are computed. The partial flow from compartment $j - 1$ across the inner border of compartment j, for example, is taken as the flow for a concentration change of c_{j-1} to 0 in the distance Δl, where the concentrations represent volume averages and the compartments are of width Δl. The accounting is done on the basis of mass; hence, the mentioned flow from compartment $j - 1$, for example, will lower the concentration in compartment $j - 1$ more than it increases the concentration in the larger compartment j. Each compartment is considered both as a recipient and as a donor in order for the final accounting to provide the net transfer.

Fick's first law gives the net flow of mass per unit area per unit time as $-D$ grad c,

in the notation of the introduction. Use this to express the partial mass influx F_{j-1} through the inner border of compartment j in time Δt for a gel of thickness h as

$$F_{j-1} = D(c_{j-1}/\Delta l)[2\pi(j-1)(\Delta l)h]\Delta t, \tag{19}$$

where $2\pi(j-1)(\Delta l)h$ is the cross-sectional area and $c_{j-1}/\Delta l$ is the gradient. This influx will change the concentration in compartments j and $j-1$ by

$$\begin{aligned}(\Delta c_j)_{j-1} &= +F_{j-1}/[\pi(2j-1)h(\Delta l)^2],\\ (\Delta c_{j-1})_{j-1} &= -F_{j-1}/[\pi(2j-3)h(\Delta l)^2],\end{aligned} \tag{20}$$

where the denominators are the volumes involved. Similarly, the partial mass influx F_j through the outer border of compartment j is

$$F_j = D(2\pi j)c_{j+1}(h\Delta l\Delta t/\Delta l), \tag{21}$$

with the resulting concentration changes of

$$\begin{aligned}(\Delta c_j)_j &= +F_j/[\pi(2j-1)h(\Delta l)^2],\\ (\Delta c_{j+1})_j &= -F_j/[\pi(2j+1)h(\Delta l)^2].\end{aligned} \tag{22}$$

The over-all accounting can be seen more easily by considering compartments from the second through the next to last and using FORTRAN notation (McCraken and Dorn, 1964). Let INDEX = 1 represent the inner border, INDEX = 2 the outer border, JJ the flanking ring from which the flow comes, and CØLD(J) the old value of C(J) at the start of the update period (the clerical function FLØAT supplies a decimal point to a number that does not have one expressed)

```
      DØ 30 J = 1, LAST
30    CØLD(J) = C(J)
      JPRIME = LAST - 1
      DØ 40 J = 2, JPRIME
      VØLJ = FLØAT (2*J - 1)
      DØ 40 INDEX = 1, 2
      JJ = J - 3 + (2*INDEX)
      VØLJJ = FLØAT (2*JJ - 1)
      AREA = 2.* FLØAT (J - 2 + INDEX)
      F = DD * AREA * CØLD(JJ)
      C(J) = C(J) + (F/VØLJ)
40    C(JJ) = C(JJ) - (F/VØLJJ).
```
(23)

DD is $D\Delta t/(\Delta l)^2$ and the common factor πh has been omitted in AREA, VØLJ, and VØLJJ because it cancels. For the linear case, AREA, VØLJ, and VØLJJ are all unity.

For the first and the last compartments there is flow through only one border of

each. Hence,

```
F = DD * 2. * CØLD(2)
C(1) = C(1) + F
C(2) = C(2) - F/3.
F = DD * 2.* FLØAT (JPRIME) * CØLD(JPRIME)
C(LAST) = C(LAST) + F/FLØAT (2*LAST - 1)
C(JPRIME) = C(JPRIME) - F/FLØAT (2*JPRIME - 1).        (24)
```

Relationship to Fick's Second Law

For constant D and cylindrical coordinates Fick's second law becomes

$$\frac{\partial c}{\partial t} = D\left[\frac{\partial^2 c}{\partial l^2} + \frac{1}{l}\left(\frac{\partial c}{\partial l}\right)\right]. \tag{25}$$

Introduce c^- and c^+ as the concentrations at $l - \Delta l$ and $l + \Delta l$, respectively. Then $\partial c/\partial l \approx (c^+ - c^-)/(2\Delta l)$ and $\partial^2 c/\partial l^2 \approx [(c^+ - c)/\Delta l - (c - c^-)/\Delta l]/\Delta l$. Combine these and regroup terms to give (Crank, 1956, p. 197).

$$D\left[\frac{\partial^2 c}{\partial l^2} + \frac{1}{l}\left(\frac{\partial c}{\partial l}\right)\right] \approx D\left[\frac{(2l - \Delta l)c^- - 4lc + (2l + \Delta l)c^+}{2l(\Delta l)^2}\right]. \tag{26}$$

(An appropriately modified formula was given by Crank for the first ring where $< \Delta l$.)

In order to show that the algorithm of equation 23 reduces to equation 26, note that the net change in concentration Δc_j for any ring j will be the sum of four contributions: one when $J = j - 1$ and INDEX = 2, two when $J = j$ for both values of INDEX, and one when $J = j + 1$ and INDEX = 1. By tracing these through in equation 23, the change in concentration per time period, using regular notation, becomes

$$\begin{aligned}\frac{\Delta c_j}{\Delta t} &= \left\{\left[-\frac{2(j-1)}{2j-1}\right]c_j + \left[\frac{2(j-1)}{2j-1}\right]c_{j-1} + \left[\frac{2j}{2j-1}\right]c_{j+1}\right. \\ &\quad \left. - \left[\frac{2j}{2j-1}\right]cj\right\}\frac{D}{(\Delta l)^2} \\ &= \{[2(j-1)c_{j-1} - (4j-2)c_j + 2jc_{j+1}]/(2j-1)\}D/(\Delta l)^2. \end{aligned} \tag{27}$$

The set-up of the problem in Fig. 1 G is such that the concentration c_j refers to the ring for which j is the normalized radius, not to the center, but to the outer border Thus,

$$j\Delta l = l + \Delta l/2. \tag{28}$$

Equations 26 and 27 can be shown to be identical by substitution of equation 28.

In a similar manner, the complicated expressions of Cox (1965) for the right-hand side of equation 25 can also be shown to be equivalent when Δl is constant.

The algorithm of equation 23 replaces the value the concentration had at the start of the computation by that value plus all the correction terms. Since the "old" values of c are used for making the update calculation, this is known as the "forward difference approximation" to the time derivative. Together with the approximation used for the spacial derivative, the over-all description of the numerical integration method is a "five-point stencil" in the notation of McCracken and Dorn (1964). This algorithm is also equivalent to that given by Watt (1968) for dispersal of animals, which was written in terms of "concentration differences" with a test for skipping, if negative, so that all elements would be counted properly.

Theoretical Limit on Mesh Size

For the parabolic differential equation 1, $\partial c/\partial t = D(\partial^2 c/\partial l^2)$, a *mesh parameter* λ has been defined as

$$\lambda = D\Delta t/(\Delta l)^2, \tag{29}$$

where Δl is the compartment size and Δt the time step. Milne (1970) explains why the five-point stencil method for numerical approximations has an upper limit of one-half for λ. Here, it can be seen in the algorithm of equation 23 that DD is λ and represents the fraction of the concentration of any one compartment distributed to a neighboring compartment. With partial flows going to both neighbors, it is reasonable that the fraction to each should not exceed one-half. The fact that the choice of the space interval Δl places a restriction on the size of the time interval Δt, because of a limit on λ, is fundamental. Even so, numerical results do not always conform to analytical results, as shown in the next section.

Test of Algorithm

In Table IV are given some results for free diffusion from a cylinder. This necessary but insufficient test was chosen because an analytical solution exists and graphs have been given for certain ranges of the parameters (Crank, 1956; Aladjem, 1971). The exact solution, for any distance l from the axis of the cylinder, is

$$c/c^0 = [1/(2Dt)] \exp[-l^2/(4Dt)] \int_0^L \exp[-l'^2/(4Dt)] I_0[ll'/(2Dt)] l' \, dl', \tag{30}$$

where I_0 is the Bessel function of the second kind of order zero and the cylinder of radius L contained all the solute at concentration c^0 at $t = 0$. This equation can be written in terms of just the two parameters

$$\alpha = (Dt/L^2)^{1/2},$$
$$\beta = l/L, \tag{31}$$

TABLE IV

TEST OF ALGORITHM FOR FREE DIFFUSION FROM INSTANTANEOUS SOURCE DEPOSITED IN CYLINDER

Annular region*	Reduced time‡, α				
	1/4	1/2	1	2	4
1	8.8(−1)§	5.5(−1)	2.1(−1) 2.2 2.1	6.0(−2)	1.58(−2)
2	5.7(−2)	1.5	1.3	5.3	1.53
3	1.7(−5)	9.5(−3) 9.4	5.5(−2) 5.5 5.6	4.2	1.45
4		1.2(−4) 1.0	1.5	2.9	1.35
5		3.0(−7) 2.0	2.5(−3) 2.4 2.4	1.8	1.22
6			2.6(−4) 2.6 2.4	9.7(−3)	1.09
7			1.8(−5) 1.7 1.4	4.7	9.74(−3)
8			7.8(−7) 7.1 4.9	2.0	8.76
Mesh size λ‖	0.030625	0.030625 0.122500	0.030625 0.122500 0.490000	0.1225 0.4900	0.49

Entries are the concentration of the middle subdivision in each annular region compared with the intial concentration inside cylinder for various mesh sizes.

* Cylinder of radius L is region No. 1; it contained initially all the solute at concentration c^0; each successive region is of width L but containing seven subdivisions.

‡ $\alpha = (Dt/L^2)^{1/2}$.

§ c/c^0 is middle element of 7; entry is to be multiplied by 10 to the power indicated in parenthesis where given, otherwise by the power given in the same column above. Individual values refer to the mesh size listed below in the same order; where a single entry appears all values were the same to two significant figures.

‖ $\lambda = D\Delta t/(\Delta l)^2$.

and so various choices of mesh size can be compared directly by expressing the results as c/c^0 at various radii relative to L for successive reduced times α. In order to simplify the presentation in Table IV and V, annular regions are defined: region No. 1 is the cylinder of radius L that initially contained all the solute at concentration c^0 and each successive region is of width L. The center of each region then corresponds to $\beta = 0.5, 1.5, 2.5 \ldots$. The computational compartments, of width Δl, have been chosen as subdivisions of each region.

Table IV gives the concentration computed for the middle subdivision when seven subdivisions per region were selected. The values of 0.88 and 0.057 check the figure given by Crank (1956, Fig. 32, p. 29) for $\alpha = \frac{1}{4}$. Surprisingly, the values in the third column for $\alpha = \frac{1}{2}$ did not check, and, on recomputing the exact solution of equation 30, Crank's figure was found to be in error. The values for $\alpha = 1$ again check Crank's figure and so the programming was considered correct and the effect of mesh size could be studied.

Table IV gives for each annular region the results with three different mesh sizes listed at the bottom in the same respective order as the entries. When there is a single entry the various values were not significantly different at the number of figures given. At large distances from the initial cylinder, say in region 8, there is an appreciable effect. For example, at $\alpha = 1$ and $\lambda = 0.49$, c/c^0 is 4.9 (10^{-7}) instead of 7.8 (10^{-7}), but at four times the time ($\alpha = 2$) there is no difference in the computed result in all regions listed.

Turn now to the effect of decreasing the number of subdivisions of the initial cylindrical source. In order to make the comparison, the average concentration for each annular region was computed by multiplying by the volume in each subdivision, summing, and dividing by the total volume. The results are given in Table V for 7, 5, 3, and 1 subdivision for two early times ($\alpha = \frac{1}{4}$ and $\frac{1}{2}$). Both the mesh size and the (redundant) number of computation cycles are given for reference. The relationship between the number of cycles $t/\Delta t$ and the other parameters is, from equations 29 and 31,

$$t/\Delta t = (\alpha^2/\lambda)(L/\Delta l)^2. \tag{32}$$

It can be seen that the number of cycles, say 100, corresponds to progressively smaller values of λ as the number of subdivisions is decreased. Even with the consequent exceedingly small values of λ, the computed average concentration varies considerably (compare the first row of each group in Table V). It is especially evident that with a coarse geometrical grid (one subdivision) too much material gets out far too fast, and that increasing the number of cycles (by reducing Δt) maintains the wrong value.

For values of $\lambda > \frac{1}{2}$, not shown, the computer output contained alternating negative and positive values of concentration. The limit of $\lambda = \frac{1}{2}$ is thus clearly

TABLE V

LIMITATION OF BROWNIAN MOTION ALGORITHM FOR FREE DIFFUSION FROM INSTANTANEOUS SOURCE DEPOSITED IN CYLINDER

Subdivisions*	Mesh‡ size λ	Computation§ cycles	Reduced time‖, α = 1/4				Computation§ cycles	Reduced time‖, α = 1/2				
			Annular region*					Annular region*				
			1	2	3	4		1	2	3	4	5
7	0.030625	100	7.3 (−1)¶	9.0 (−2)	1.7 (−4)	3.6 (−9)	400	4.8 (−1)	1.5 (−1)	1.3 (−2)	2.4 (−4)	1.0 (−6)
5	0.015625	100	7.3	8.8	2.1	1.7 (−8)	400	4.8	1.5	1.3	2.6	1.4
	0.031250	50	7.3	8.8	2.0	1.2	200	4.8	1.5	1.3	2.5	1.3
	0.156250	10	7.3	9.0	1.1	0	40	4.8	1.5	1.3	2.2	6.5 (−7)
3	0.005625	100	7.6	7.9	3.2	1.9 (−7)	400	4.9	1.5	1.2	3.2	3.2 (−6)
	0.011250	50	7.6	8.0	3.1	1.6	200	4.9	1.5	1.2	3.1	3.1
	0.056250	10	7.6	8.1	2.2	2.5 (−8)	40	4.9	1.5	1.2	2.8	2.0
1	0.000625	100	8.8	3.7	9.1	1.6 (−5)	400	6.3	1.0	1.0	7.3	4.0 (−5)
	0.001250	50	8.8	3.7	9.0	1.6	200	6.3	1.0	1.0	7.3	4.0
	0.006250	10	8.8	3.7	8.5	1.2	40	6.3	1.0	1.0	7.0	3.6

Entries are the average concentration in each annular region compared with the initial concentration inside cylinder.

* Cylinder of radius L is region No. 1; it contained initially all the solute at concentration c^0; each successive region is of width L but containing $L/\Delta l$ subdivisions.

‡ $\lambda = D\Delta t/(\Delta l)^2$

§ $t/\Delta t = (\alpha^2/\lambda)(L/\Delta l)^2$

‖ $\alpha = (Dt/L^2)^{1/2}$

¶ $c/c^0 = \sum(2j-1)c_j/\sum(2j-1)$, where $\sum$ extends over the number of subdivisions per region; entry is to be multiplied by 10 to the power indicated in parenthesis where given, otherwise by the power given in the same column above.

real. On close inspection, results for $\lambda = \frac{1}{2}$ were not monotonically decreasing. This is because the cylindrical geometry with its volume differences between compartments imposes an even smaller value for the fraction that can flow from one region to another for numerical stability of the computations. In connection with large values of λ, even though less than $\frac{1}{2}$, it should be noted that the entry in the fourth row, sixth column of Table V is an absolute zero: with only 10 cycles no material was computed to have moved that far. This illustrates the frequently stated limitation that results for short times near sharp changes in concentration will be in error (Crank, 1956, p. 200). The mathematical reason is that a ratio of finite differences only approximates a derivative.

In immunodiffusion there will be components of various sizes and shapes. Selection of the update period appropriately for the one with the largest D will mean that more computations are performed than necessary for the one with the smallest D. The special choice of $\lambda = \frac{1}{6}$ to minimize truncation errors (McCracken and Dorn, 1964, p. 382) cannot simultaneously hold for all components.

DISCUSSION

The Spiers-Augustin theory "fits" only for very dilute antiserum and high antigen excess. Hence, the mechanism of immunodiffusion is more complicated than merely precipitation of a compound of equivalence proportions at a moving front. The analysis presented here shows also that any attempt to include a realistic reaction mechanism in the differential equations themselves will most surely give equations that cannot be integrated.

The proposal of Augustin to alternate computation cycles of unrestricted diffusion and chemical reaction appears to offer a way of solving even the most complex set of interactions. The use of a computer to do this, however, does not automatically mean the results will be correct: care must be taken to select a very small mesh and extensive considerations of reasonableness must be made. Some of these include: (*a*) radial immunodiffusion starts with the computationally unfavorable situation of a sharp discontinuity. (*b*) Precipitate lines may perpetuate this problem. (*c*) Each species with a different D will give a *different* mesh parameter λ for the same number of cycles of computation. (*d*) As Δt is reduced, the speeds of reaction and resolubilization may enter in.

The smallest value of the reduced time used in Table V corresponds to about $\frac{1}{2}$ hr for antibody diffusing from a 1-mm radius well. The use of 50 computation cycles to reach this time represents an update about every $\frac{1}{2}$ min. Considering that plates are observed for several days, one can see the enormity of the project using this algorithm.

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