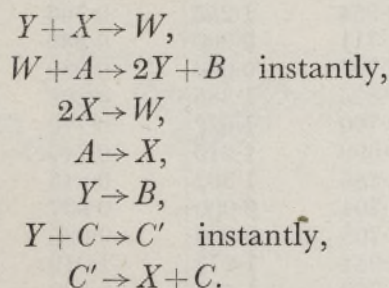


rate 5×10^{-4} of the difference of X -content of the two cells per second. In other words, if there is nothing altering the concentrations but diffusion the difference of concentrations suffers an exponential decay with time constant 1000 s, or 'half-period' of 700 s. These times are doubled for Y .

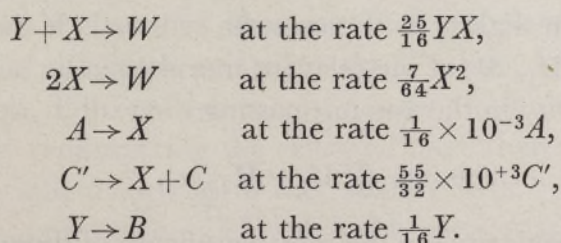
If the cell membrane is regarded as the only obstacle to diffusion the permeability of the membranes to the morphogen is 5×10^{-6} cm/s or 0.018 cm/h. Values as large as 0.1 cm/h have been observed (Davson & Danielli 1943, figure 28).

(iii) The reactions are the most important part of the assumptions. Four substances A, X, Y, B are involved; these are isomeric, i.e. the molecules of the four substances are all rearrangements of the same atoms. Substances C, C', W will also be concerned. The thermodynamics of the problem will not be discussed except to say that it is contemplated that of the substances A, X, Y, B the one with the greatest free energy is A , and that with the least is B . Energy for the whole process is obtained by the degradation of A into B . The substance C is in effect a catalyst for the reaction $Y \rightarrow X$, and may also be regarded as an evocator, the system being unstable if there is a sufficient concentration of C .

The reactions postulated are



(iv) For the purpose of stating the reaction rates special units will be introduced (for the purpose of this section only). They will be based on a period of 1000 s as units of time, and 10^{-11} mole/cm³ as concentration unit*. There will be little occasion to use any but these special units (s.u.). The concentration of A will be assumed to have the large value of 1000 s.u. and the catalyst C , together with its combined form C' the concentration $10^{-3}(1 + \gamma)$ s.u., the dimensionless quantity γ being often supposed somewhat small, though values over as large a range as from -0.5 to 0.5 may be considered. The rates assumed will be



With the values assumed for A and C' the net effect of these reactions is to convert X into Y at the rate $\frac{1}{32}[50XY + 7X^2 - 55(1 + \gamma)]$ at the same time producing X at the constant rate $\frac{1}{16}$, and destroying Y at the rate $Y/16$. If, however, the concentration of Y is zero and the rate of increase of Y required by these formulae is negative, the rate of conversion of Y into X is reduced sufficiently to permit Y to remain zero.

* A somewhat larger value of concentration unit (e.g. 10^{-9} mole/cm³) is probably more suitable. The choice of unit only affects the calculations through the amplitude of the random disturbances.

In the special units $\mu = \frac{1}{2}$, $\nu = \frac{1}{4}$.

(v) Statistical theory describes in detail what irregularities arise from the molecular nature of matter. In a period in which, on the average, one should expect a reaction to occur between n pairs (or other combinations) of molecules, the actual number will differ from the mean by an amount whose mean square is also n , and is distributed according to the normal error law. Applying this to a reaction proceeding at a rate F (s.u.) and taking the volume of the cell as 10^{-8} cm^3 (assuming some elongation tangentially to the ring) it will be found that the root mean square irregularity of the quantity reacting in a period τ of time (s.u.) is $0.004 \sqrt{(F\tau)}$.

TABLE 1. SOME STATIONARY-WAVE PATTERNS

| cell number | first specimen | | | | second specimen: incipient Y | 'slow cooking': incipient Y | four-lobed equilibrium | |
|----------------|-------------------|-------|---------------|-------|---------------------------------------|--------------------------------------|------------------------|-------|
| | incipient pattern | | final pattern | | | | X | Y |
| | X | Y | X | Y | | | | |
| 0 | 1.130 | 0.929 | 0.741 | 1.463 | 0.834 | 1.057 | 1.747 | 0.000 |
| 1 | 1.123 | 0.940 | 0.761 | 1.469 | 0.833 | 0.903 | 1.685 | 0.000 |
| 2 | 1.154 | 0.885 | 0.954 | 1.255 | 0.766 | 0.813 | 1.445 | 2.500 |
| 3 | 1.215 | 0.810 | 1.711 | 0.000 | 0.836 | 0.882 | 0.445 | 2.500 |
| 4 | 1.249 | 0.753 | 1.707 | 0.000 | 0.930 | 1.088 | 1.685 | 0.000 |
| 5 | 1.158 | 0.873 | 0.875 | 1.385 | 0.898 | 1.222 | 1.747 | 0.000 |
| 6 | 1.074 | 1.003 | 0.700 | 1.622 | 0.770 | 1.173 | 1.685 | 0.000 |
| 7 | 1.078 | 1.000 | 0.699 | 1.615 | 0.740 | 0.956 | 0.445 | 2.500 |
| 8 | 1.148 | 0.896 | 0.885 | 1.382 | 0.846 | 0.775 | 0.445 | 2.500 |
| 9 | 1.231 | 0.775 | 1.704 | 0.000 | 0.937 | 0.775 | 1.685 | 0.000 |
| 10 | 1.204 | 0.820 | 1.708 | 0.000 | 0.986 | 0.969 | 1.747 | 0.000 |
| 11 | 1.149 | 0.907 | 0.944 | 1.273 | 1.019 | 1.170 | 1.685 | 0.000 |
| 12 | 1.156 | 0.886 | 0.766 | 1.451 | 0.899 | 1.203 | 0.445 | 2.500 |
| 13 | 1.170 | 0.854 | 0.744 | 1.442 | 0.431 | 1.048 | 0.445 | 2.500 |
| 14 | 1.131 | 0.904 | 0.756 | 1.478 | 0.485 | 0.868 | 1.685 | 0.000 |
| 15 | 1.090 | 0.976 | 0.935 | 1.308 | 0.919 | 0.813 | 1.747 | 0.000 |
| 16 | 1.109 | 0.957 | 1.711 | 0.000 | 1.035 | 0.910 | 1.685 | 0.000 |
| 17 | 1.201 | 0.820 | 1.706 | 0.000 | 1.003 | 1.050 | 0.445 | 2.500 |
| 18 | 1.306 | 0.675 | 0.927 | 1.309 | 0.899 | 1.175 | 0.445 | 2.500 |
| 19 | 1.217 | 0.811 | 0.746 | 1.487 | 0.820 | 1.181 | 1.685 | 0.000 |

The diffusion of a morphogen from a cell to a neighbour may be treated as if the passage of a molecule from one cell to another were a monomolecular reaction; a molecule must be imagined to change its form slightly as it passes the cell wall. If the diffusion constant for a wall is μ , and quantities M_1 , M_2 of the relevant morphogen lie on the two sides of it, the root-mean-square irregularity in the amount passing the wall in a period τ is

$$0.004 \sqrt{\{(M_1 + M_2) \mu \tau\}}.$$

These two sources of irregularity are the most significant of those which arise from truly statistical cause, and are the only ones which are taken into account in the calculations whose results are given below. There may also be disturbances due to the presence of neighbouring anatomical structures, and other similar causes. These are of great importance, but of too great variety and complexity to be suitable for consideration here.

(vi) The only morphogen which is being treated as an evocator is C . Changes in the concentration of A might have similar effects, but the change would have to be rather great. It is preferable to assume that A is a 'fuel substance' (e.g. glucose) whose concentration does

not change. The concentration of C , together with its combined form C' , will be supposed the same in all cells, but it changes with the passage of time. Two different varieties of the problem will be considered, with slightly different assumptions.

The results are shown in table 1. There are eight columns, each of which gives the concentration of a morphogen in each of the twenty cells; the circumstances to which these concentrations refer differ from column to column. The first five columns all refer to the same 'variety' of the imaginary organism, but there are two specimens shown. The specimens differ merely in the chance factors which were involved. With this variety the value of γ was allowed to increase at the rate of 2^{-7} s.u. from the value $-\frac{1}{4}$ to $+\frac{1}{16}$. At this point a pattern had definitely begun to appear, and was recorded. The parameter γ was then allowed to decrease at the same rate to zero and then remained there until there was no

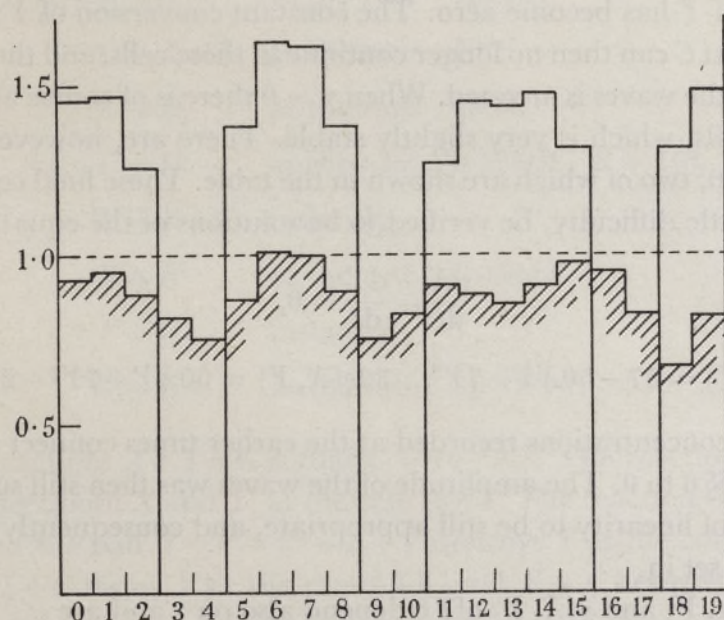


FIGURE 3. Concentrations of Y in the development of the first specimen (taken from table 1).
 ----- original homogeneous equilibrium; ///// incipient pattern; ——— final equilibrium.

more appreciable change. The pattern was then recorded again. The concentrations of Y in these two recordings are shown in figure 3 as well as in table 1. For the second specimen only one column of figures is given, viz. those for the Y morphogen in the incipient pattern. At this stage the X values are closely related to the Y values, as may be seen from the first specimen (or from theory). The final values can be made almost indistinguishable from those for the first specimen by renumbering the cells and have therefore not been given. These two specimens may be said to belong to the 'variety with quick cooking', because the instability is allowed to increase so quickly that the pattern appears relatively soon. The effect of this haste might be regarded as rather unsatisfactory, as the incipient pattern is very irregular. In both specimens the four-lobed component is present in considerable strength in the incipient pattern. It 'beats' with the three-lobed component producing considerable irregularity. The relative magnitudes of the three- and four-lobed components depend on chance and vary from specimen to specimen. The four-lobed component may often be the stronger, and may occasionally be so strong that the final pattern is four-lobed. How often this happens is not known, but the pattern, when it occurs, is shown in the last

two columns of the table. In this case the disturbances were supposed removed for some time before recording, so as to give a perfectly regular pattern.

The remaining column refers to a second variety, one with 'slow cooking'. In this the value of γ was allowed to increase only at the rate 10^{-5} . Its initial value was -0.010 , but is of no significance. The final value was 0.003 . With this pattern, when shown graphically, the irregularities are definitely perceptible, but are altogether overshadowed by the three-lobed component. The possibility of the ultimate pattern being four-lobed is not to be taken seriously with this variety.

The set of reactions chosen is such that the instability becomes 'catastrophic' when the second-order terms are taken into account, i.e. the growth of the waves tends to make the whole system more unstable than ever. This effect is finally halted when (in some cells) the concentration of Y has become zero. The constant conversion of Y into X through the agency of the catalyst C can then no longer continue in these cells, and the continued growth of the amplitude of the waves is arrested. When $\gamma = 0$ there is of course an equilibrium with $X = Y = 1$ in all cells, which is very slightly stable. There are, however, also other stable equilibria with $\gamma = 0$, two of which are shown in the table. These final equilibria may, with some trouble but little difficulty, be verified to be solutions of the equations (6.1) with

$$\frac{dX}{dt} = \frac{dY}{dt} = 0,$$

$$\text{and} \quad 32f(X, Y) = 57 - 50XY - 7Y^2, \quad 32g(X, Y) = 50XY + 7Y^2 - 2Y - 55.$$

The morphogen concentrations recorded at the earlier times connect more directly with the theory given in §§ 6 to 9. The amplitude of the waves was then still sufficiently small for the approximation of linearity to be still appropriate, and consequently the 'catastrophic' growth had not yet set in.

The functions $f(X, Y)$ and $g(X, Y)$ of § 6 depend also on γ and are

$$f(X, Y) = \frac{1}{32}[-7X^2 - 50XY + 57 + 55\gamma],$$

$$g(X, Y) = \frac{1}{32}[7X^2 + 50XY - 2Y - 55 - 55\gamma].$$

In applying the theory it will be as well to consider principally the behaviour of the system when γ remains permanently zero. Then for equilibrium $f(X, Y) = g(X, Y) = 0$ which means that $X = Y = 1$, i.e. $h = k = 1$. One also finds the following values for various quantities mentioned in §§ 6 to 9:

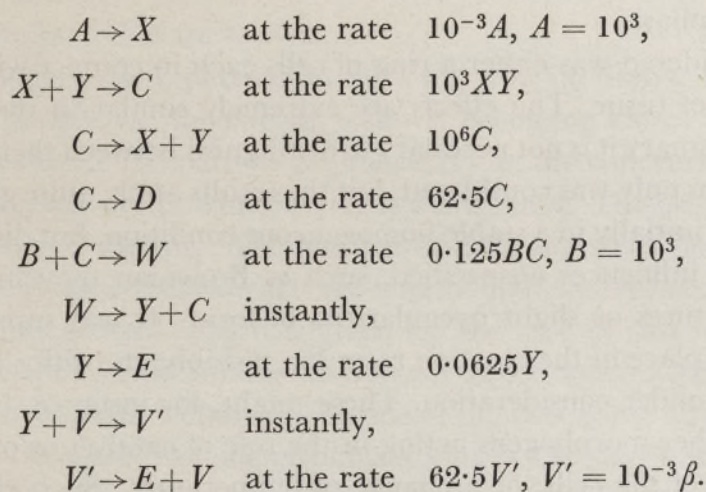
$$\begin{aligned} a &= -2, \quad b = -1.5625, \quad c = 2, \quad d = 1.500, \quad s = 3.333, \\ I &= 0, \quad \alpha = 0.625, \quad \chi = 0.500, \quad (d-a)(-bc)^{-\frac{1}{2}} = 1.980, \\ (\mu + \nu)(\mu\nu)^{-\frac{1}{2}} &= 2.121, \quad p_0 = -0.25 \pm 0.25i, \\ p_2 &= -0.0648, \quad p_3 = -0.0034, \quad p_4 = -0.0118. \end{aligned}$$

(The relation between p and U for these chemical data, and the values p_n , can be seen in figure 1, the values being so related as to make the curves apply to this example as well as that in § 8.) The value $s = 3.333$ leads one to expect a three-lobed pattern as the commonest, and this is confirmed by the values p_n . The four-lobed pattern is evidently the closest competitor. The closeness of the competition may be judged from the difference $p_3 - p_4 = 0.0084$,

which suggests that the three-lobed component takes about 120 s.u. or about 33 h to gain an advantage of a neper (i.e. about 2.7:1) over the four-lobed one. However, the fact that γ is different from 0 and is changing invalidates this calculation to some extent.

The figures in table 1 were mainly obtained with the aid of the Manchester University Computer.

Although the above example is quite adequate to illustrate the mathematical principles involved it may be thought that the chemical reaction system is somewhat artificial. The following example is perhaps less so. The same 'special units' are used. The reactions assumed are



The effect of the reactions $X + Y \rightleftharpoons C$ is that $C = 10^{-3}XY$. The reaction $C \rightarrow D$ destroys C , and therefore in effect both X and Y , at the rate $\frac{1}{16}XY$. The reaction $A \rightarrow X$ forms X at the constant rate 1, and the pair $Y + V \rightarrow V' \rightarrow E + V$ destroys Y at the constant rate $\frac{1}{16}\beta$. The pair $B + C \rightarrow W \rightarrow Y + C$ forms Y at the rate $\frac{1}{8}XY$, and $Y \rightarrow E$ destroys it at the rate $\frac{1}{16}Y$. The total effect therefore is that X is produced at the rate $f(X, Y) = \frac{1}{16}(16 - XY)$, and Y at the rate $g(X, Y) = \frac{1}{16}(XY - Y - \beta)$. However, $g(X, Y) = 0$ if $Y \leq 0$. The diffusion constants will be supposed to be $\mu = \frac{1}{4}$, $\nu = \frac{1}{16}$. The homogeneity condition gives $hk = 16$, $k = 16 - \beta$.

It will be seen from conditions (9.4a) that case (d) applies if and only if $\frac{4}{k} + \frac{k}{4} < 2.75$, i.e. if k lies between 1.725 and 9.257. Condition (9.4b) shows that there will be instability if in addition $\frac{8}{k} + \frac{k}{8} > \sqrt{3} + \frac{1}{2}$, i.e. if k does not lie between 4.98 and 12.8. It will also be found that the wave-length corresponding to $k = 4.98$ is 4.86 cell diameters.

In the case of a ring of six cells with $\beta = 12$ there is a stable equilibrium, as shown in table 2.

TABLE 2

| cell | 0 | 1 | 2 | 3 | 4 | 5 |
|------|-----|-----|-----|-----|-----|-----|
| X | 7.5 | 3.5 | 2.5 | 2.5 | 3.5 | 7.5 |
| Y | 0 | 8 | 8 | 8 | 8 | 0 |

It should be recognized that these equilibria are only dynamic equilibria. The molecules which together make up the chemical waves are continually changing, though their concentrations in any particular cell are only undergoing small statistical fluctuations. Moreover,