Population Growth in Planaria

*Dugesia tigrina* (Gerard)

*Regulation by the absolute number in the population*

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**Abstract** Planaria reproduce by transverse fission. Isolated worms increase in number exponentially, while social animals at the same density are inhibited in terms of numerical increase, but over a 25 day period undergo a larger increase in mass. Isolated posterior fission products reproduce faster than isolated anterior fission products. Regulation of population growth is independent of density over a 16-fold range and regulatory factors cannot be demonstrated in the medium. Regulation of population growth depends on direct contact between animals. Fission period varies from individual to individual and from period to period for a given individual. Doubling time is related to the absolute number of individuals comprising the population as follows:

\[ P_N = \frac{(P_M \cdot N)}{(K + N)}, \]

where \( P_N \) is the doubling period of a population of \( N \) individuals, \( P_M \) is the doubling time of an infinitely large population, \( N \) is the number of individuals in the population, and \( K \) is the number of individuals in a population the period of which is one-half \( P_M \). At 22°-24°C \( P_M \) is estimated to be 43.3 days and \( K \) is 1.87 individuals. A model system assumes that inhibitor flows through the population from animal to animal from the slowest to the fastest animal in the population thus acting to synchronize population increase as well as to determine the rate of population growth. A possible source of the inhibitor is discussed.

**Introduction**

Planaria, like other triclad turbellarian worms, reproduce asexually by transverse fission, the anterior fragment retaining the pharynx and subsequently regenerating a new tail, while the posterior fragment regenerates a new head and by reworking existing tissues (morphollaxis) reorganizes the digestive system and forms a new pharynx (4, 8).

The stock employed in this study was derived from animals received from...
Carolina Biological Supply Co. (Burlington, N. C.) in February 1971. On writing to the company I learned that the worms had been collected and shipped from southern Louisiana, U.S.A. I identified the animals as *Dugesia tigrina* (Gerard), a species widely distributed in North America (9). The animals all proved to be in the sexual phase and laid hundreds of stalked capsules (cocoons) from which worms hatched in 14–17 days. These juvenile worms and their fission products constitute the material with which the following experiments were conducted. The animals, which were all in the asexual or fissioning state, grew into large robust animals, but increased very slowly in numbers, indicating some sort of regulatory mechanism acting to inhibit the exponential potential to be expected from a binary reproductive process. Experiments were designed to elucidate the mechanism of population regulation. They are presented here in the order in which they were performed during the months of March through September 1972.

**MATERIALS AND METHODS**

The laboratory was air-conditioned to maintain an air temperature of 22°–24°C, and laboratory photoperiod was fixed at 14L:10D, the lights coming on at 5 a.m. and going off at 7 p.m. EST. All light sources were "cool white" fluorescent and the intensities at the level of the animals were in the range of 50–100 foot candles (Photovolt Model 200 light meter, Photovolt Corp., New York). The medium was tap water (from Lake Champlain) drawn from the main input into the Marsh Life Science building and aerated vigorously for 24 h before use. As in an earlier study (3) water drawn from the main input to the building proved to be an excellent culture medium, while the same water drawn from the laboratory tap was toxic, probably due to heavy metals acquired during passage through soldered copper pipes. The worms were maintained before each experiment in 200-mm stacking dishes containing from 50 to 150 animals in 600 ml of medium. The animals were fed beef kidney cut in thin slices and left with the animals for 3–5 h, after which the kidney was removed and the medium replaced. Animals having just fed would not feed the following day, but a substantial fraction of the animals would feed on an alternate day basis. To ensure that food supply was not a limiting factor, the alternate day schedule of feeding and changing was continued throughout the study for both stock and experimental material. As the animals increased in numbers they were subcultured into additional stacking dishes. All manipulations were carried out in the light. Unless noted otherwise, each experiment was initiated with intact worms drawn from stock cultures. A daily record was kept of the numerical status of all experimental populations. At the termination of the first experiment, an estimate of the biomass of the animals was made by photographing the living animals and subsequently projecting the images on standard weight reference cards. The outlines of the animals were cut out along with a square constructed from a known linear dimension, and the areas of the animals calculated from the weights of the cutouts (Mettler Model H balance, Mettler Instrument Corp., Princeton, N.J.). Statistical procedures were taken from Snedecor and Cochran (11).
The experiments depicted in Figs. 1, 2, 3, 4, 8, 9, 10, and 11, and Tables I and III, were carried out with the vessels all loosely covered and in thermal equilibrium with the ambient air temperature of 22°-24°C. The experiments represented in Figs. 5, 6, and 7 and Table II were of necessity carried out with uncovered vessels. Water temperature for the uncovered experiments was approximately 2°C below ambient due to cooling by vaporization.

RESULTS

The Growth of Social vs. Isolated Populations

The first experiment compared the growth potential of a social population of animals with an equal initial number of individuals, maintained at the same density, but isolated and hence not subject to interaction with other worms. 20 worms were drawn at random from a stock culture. 10 of these were placed together in a 200 mm stacking dish containing 500 ml of medium. 10 others were each placed in a 50 ml beaker containing 50 ml of medium. As the group increased in number, medium was added to maintain the population density constant at 20/1. As the isolates fissioned, the posterior fission product was transferred to a 50 ml beaker containing 50 ml of medium. Cannibalism, which was restricted to the social group, was noted when it occurred. The individual lineages of each of the 10 clones was recorded. At the termination of the experiment on day 25, all the animals were photographed for the biomass estimate. Fig. 1 represents the numerical status of the two systems as a function of time. The ordinate is the cumulative total number of animals and the abscissa is time in days, the isolates indicated by
circles and the group by triangles. Four posterior fission products were can-
nibalized in the group regime, one on day 3, two on day 17, and one on day
25. The numbers for the group do not include the animals which were can-
nibalized but rather the actual numerical status of the population. On day
25 the isolates outnumbered the group 73 to 22. The increase in numbers
for the group was stepwise in form, while the numerical increase for the iso-
lates seemed to be exponential as one might expect for a system not subject
to inhibition.

In Fig. 2 the ordinate is the logarithm (base 10) of the total cumulative
number of isolates and the abscissa is time in days. The solid line connects
data points, while the dotted line was fitted by the method of least squares
utilizing all 26 data points. The equation of the dotted line is as follows:

\[
\log_{10} (\text{total number}) = 1.084 + 0.031 (\text{days}).
\]

The coefficient of determination \(R^2\) is 0.986. The antilog of 0.031 is 1.079
corresponding to a 7.9\% increase in number per day. The intercept on the
ordinate (1.084) is the log of 12.1, indicating that the population of isolates
increased exponentially as if it had started with 12.1 individuals rather than
with 10 as was the actual case.

Fig. 3 represents the outlines of all 95 animals as they were determined
from photographs taken on day 25. The size of the animals in the group is
typical of the size of the 20 animals with which the experiment was initiated.
The 10 clones are lettered A through J with the stem animal at the top of
each clone. Note that the stem animals have undergone a slight reduction
in size during the 25 day period, while the clonal products have without ex-
ception developed into smaller animals than the stem animal from which
they were derived.
Table I summarizes the total numbers produced, the combined areas of the 10 clones, the sum of those combined areas, and the combined areas of the 22 animals in the group. Notwithstanding the great numerical difference

\[
\begin{array}{ccc}
\text{Clone} & \text{Number} & \text{Combined area} \\
A & 7 & 0.230 \\
B & 7 & 0.224 \\
C & 8 & 0.224 \\
D & 8 & 0.219 \\
E & 4 & 0.149 \\
F & 10 & 0.224 \\
G & 10 & 0.230 \\
H & 9 & 0.254 \\
I & 4 & 0.170 \\
J & 6 & 0.144 \\
\hline
\text{Total number} & 73 \\
\text{Total area} & 2.07 \\
\text{Group} & 22 \\
\text{Combined area} & 2.31 \\
\end{array}
\]

between the isolates (73) and the group (22), the combined area of the group (2.31 cm\(^2\)) is larger than the combined area of the isolates (2.07 cm\(^2\)). Since the larger animals in the group are probably thicker than the smaller isolates, actual mass differences may be even larger than these figures would indicate. I suggest three possible explanations for the mass differences. First, the isolates fission at a higher frequency than the animals in the group, and the posterior fission product cannot feed for several days since it lacks a pharynx. Second, the isolates may be expending a larger percentage of available nu-
tritional energy for morphollaxis and regeneration. Third, the smaller isolated individuals may have a higher maintenance requirement than the larger animals in the group. The final numbers within each clone ranged from 4 to 10, a ratio of 2.5. The combined areas exhibit a smaller range of from 1.44 to 2.54 cm², a ratio of 1.76. The three clones with the smallest final numbers (E, I, and J) are also those clones with the smallest combined areas. Since the original material for these experiments was sexually derived, the difference between clones may have a genetic basis.

Differences are further indicated in the reproductive patterns of the 10 clones illustrated in Fig. 4. The stem animals are lettered A through J. Their posterior fission products are numbered by subscript in the order in which they were produced. Posterior products of posterior products are numbered in the same fashion. Using clone A as an example, A/1, A/2, and A/3 are the sequential products produced by the stem animal A. The first fission product of the first fission product is numbered A/11, while the second fission product of the first fission product is numbered A/12. Similarly the first fission product of the second fission product is numbered A/21. The numbers at the sides of Fig. 4 are the days of the experiment and each individual is entered on the day it was produced. The lines joining the individuals serve to further clarify the derivations of the animals. A surprising degree of variation is evident in the reproductive mode of the 10 clones. Clone E represents one extreme, in which only the stem animal produced fission products. The other extreme is indicated by clone I, in which the stem animal fissioned only once. That product in turn produced a fission product, and the latter product

![Figure 4](image-url)

Figure 4. The lineages of the 10 clones depicted in Fig. 3. Each animal is entered on the day of the experiment it was produced. Note the variation in reproductive mode among the 10 clones. A statistical analysis of the fission histories appears in the text.
then produced a fission product. The remaining clones reproduced by combinations of the two modes. In clones B, C, F, G, H, and I it was typical for the posterior product to fission before it completed head regeneration.

The fission period of the stem animals is defined as the time elapsed between successive fissionings. Individual I is not included in these calculations as it fissioned only once. 18 values read from Fig. 4 gave a mean and standard deviation of 9.94 ± 1.95 days. The fission period of the posterior product is defined as the time elapsed between the time of its production and the time of its subsequent fission. 28 values read from Fig. 4 gave a mean and SD of 8.25 ± 2.65 days. The Student’s t test for groups of unequal size was applied to the data, yielding a t value of 2.27, which for 44 degrees of freedom, corresponds to a P value of 0.028. Posterior products thus fission at a significantly (0.028 level) higher rate than anterior (stem) products. Lender and Zghal (6) have reached a similar although nonstatistical conclusion with the European species D. gonocephala. Also, a simple decapitation induces fissioning in D. dorotocephala (1, 2). These authors agree in assigning an inhibitory role to the brain. The idea is attractive since the posterior fission product has no brain, so inhibition would be minimal until the brain is reformed.

On the completion of the first experiment, the following conclusions were drawn. Under conditions of identical numerical concentration, isolated worms multiply in an uninhibited and exponential fashion, while worms interacting as a population are inhibited in terms of numerical increase, but over a 25 day period undergo a larger absolute increase in mass. The interaction among social animals is thus in no sense energetically wasteful.

Are Regulatory Factors Liberated into the Medium?

The next experiments were designed to test for activating or inhibiting products elaborated by animals in the isolated or group regimes. The inhibited state of social animals was not relieved by continuous aeration or mechanical stirring of the medium. Neither the medium nor the substrate tested separately or together proved to be inhibitory to isolated individuals when preconditioned by crowded social populations. Similarly, media preconditioned by isolated individuals failed to activate fissioning in social populations. The possibility remained that interactions might be mediated by factors with very short half-life as has been proposed by Rose and Rose (10) for tadpoles and fish.

The first experiment was designed to see if a particular stage in the fissioning cycle might be accompanied by the release of an inhibitor into the medium. 40 worms were removed from a stock population. 20 of these were placed together in a 200 mm stacking dish containing 800 ml of medium. The dish was continuously and gently aerated to mix the contents. The remaining 20 animals were each placed in 50-ml beakers containing 40 ml
of medium. 19 of the beakers were joined by inverted glass U tubes (5 mm ID) containing medium. The medium was lifted from the 19th to the 20th beaker by a gentle air stream liberated within an inverted glass J tube, resulting in an equilibrium head of about 18 mm of water driving the medium around the circular array of beakers at a flow rate of about 8 ml/min or about 60% of the total volume/h. It was necessary to block each U tube at one end with a loose tuft of polyester fiber to prevent worms from migrating from beaker to beaker and thus forming aggregates. Separate experiments demonstrated that the migrations were against the slow current but took place only if the incurrent medium was conditioned by worms. The migrations were thus not rheokinetic but rather chemokinetic in nature. This was an important point to establish since social animals formed spontaneous aggregates usually just below the air-water interface on the vertical walls of the container. The formation of such aggregates was clearly not simply the result of chance encounters, but rather resulted from a directed attraction between animals. Both the isolated and group regimes ran continuously except for a period of 3–5 h on alternate days during which the animals were fed and the media replaced. Posterior fission products were removed and recorded daily. The experiment ran for 25 days. On day 15 the regimes were reversed. The 20 animals previously in the group were isolated in beakers, and the 20 previously isolated were placed together in a 200 mm stacking dish. Fig. 5 summarizes the experiment. The ordinate is the total cumulative number of posterior fission products and the abscissa is time in days. The isolates are represented by circles and the group by triangles. Note the change of symbols on day 15 when the regimes were reversed. By day 15 the isolates had outproduced the group 23 to 8. Within 24 h of isolation the original group produced 12 fission products. By day 25 the original group, now

![Figure 5](image-url)

**Figure 5.** The total number of fission products produced as a function of time for 20 isolated animals sharing the same medium (circles) and 20 animals together as a population (triangles). Note the change in symbols on day 15 when the regimes were reversed. Density was maintained constant throughout at 25/1.
isolated, had outproduced the original isolates 31 to 24. The experiment failed to demonstrate any inhibitory factors transmitted via the medium. What it did demonstrate was that social animals tend to synchronize and accumulate in a prefission state. Further, isolated animals are immediately subject to inhibition when placed together as a population.

The possibility still remained that social animals might be liberating an inhibitor into the medium. This possibility was explored in the next experiment which was identical in design with the last experiment except that the medium was shared by all the animals. The 20 beakers and the stacking dish were all joined in continuous circular series with U tubes, the medium flowing at about 8 ml/min as before. The results appear in Fig. 6. Note the stepwise increase in fission products produced by the group as in the experiment previous. At the termination of the experiment on day 15, the isolates had produced 32 fission products compared to 11 for the group, a ratio of 2.91, comparable to the ratio of 2.88 (23 to 8) for the same time period in the previous experiment. These two experiments failed to indicate any regulatory role for products transmitted through the medium.

*The Effect of Absolute Number in the Population on Fission Rate*

Observations with a horizontal microscope revealed that aggregated animals were in direct cellular contact chiefly along their lateral margins. The simplest hypothesis was that information was transmitted from animal to animal while they were in the aggregated state. Since aggregation requires two or more animals, the following experiment was designed to estimate the effect of absolute population size on fission rate under conditions of constant density. The number of animals in each population set was partially dictated by available glassware which was selected to provide approximately the same water depth (and hence air surface per worm) for each population. The animals were drawn from stock cultures and randomly distributed as follows:

![Figure 6](image_url)

*Figure 6.* The experiment is identical with Fig. 5 except that the medium was shared by all the animals.
12 individuals each isolated in 40 ml; six pairs each in 80 ml; three groups of four each in 160 ml; one group of 12 in 480 ml; one group of 24 in 960 ml. For these population sets the experiment was performed in triplicate. The fission rate was also determined for 50/2 1 and 100/4 1, but due to the large number of animals required the experiment was done only once. Thus 366 animals were used in all. The fission rate was arbitrarily chosen as the percentage of the animals in each population set which had produced fission products within 4 days. Fission products were removed daily to maintain population densities constant throughout at 25/1. The results appear in Fig. 7. The ordinate is the percentage of the animals in each population set which fissioned within 4 days. The abscissa, which is distributed logarithmically for convenience, is the absolute size of each population, or the number of animals in the dish. The solid line in Fig. 7 connects mean fission rates for each of the seven population sizes. The rates are as follows: 78% for singles; 44% for pairs; 22% for groups of 4; 2.8% for groups of 12; 7% for groups of 24; 6% for the group of 50; 5% for the group of 100. The fission rate for singles is much higher than the steady-state fission rate (7.9%/day) calculated for isolates in Fig. 2. However, it is typical for worms to undergo a rapid increase in numbers immediately after isolation from a social population (Figs. 1, 2, 5, and 6). Thus what this experiment illustrates is the short-term effect of population size on the fission rate of animals previously in a social regime. Note in Fig. 7 that fission rate does not approach zero as population size increases, but rather approaches a base fission rate estimated to be about 5%.

Figure 7. The percent of animals fissioning within 4 days after removal from a prior social regime (ordinate) as a function of the absolute number in each population set (abscissa). The abscissa is distributed logarithmically for convenience. The solid line connects the mean values for the data points. The dotted line connects values calculated from the empirical equation derived in the text. Density is constant throughout at 25/1.
In an attempt to simulate the observed results, the following equation was developed utilizing two experimentally estimated rate constants:

\[
\text{Fission rate} = k_1 + (k_2 - k_1) \cdot (1/N),
\]

where fission rate is the percentage of the population fissioning within 4 days, \(k_1\) is the base fission rate estimated to be about 5\%, \(k_2\) is the fission rate of the isolated individuals (78\%), and \(N\) is the number of individuals in the population.

The equation states that the observed rate of fissioning is the sum of two rates. The first of these is a constant, independent of population size, while the second rate is inversely proportional to the number of individuals in the population. The dotted line in Fig. 7 connects points calculated from the above equation, which fairly well approximates the observed values for population sizes of 1 to 100. It should be noted that the assumption of a basal rate greater than zero is mandatory, since stock animals maintained at higher concentrations and numbers than those employed in this experiment nevertheless continue to feed actively and accordingly must fission at some basal rate.

**Fission Rate is Independent of Density**

The experiments previously described were each carried out under conditions of constant density, the first experiment at 20/1, and the remaining at 25/1. The following experiment was designed to evaluate the effect of density, if any, on the reproductive rates of isolated and social populations. I selected three concentrations for the analysis. The first was 25/1 as employed previously. The second was 4 times that concentration (100/1) and the third was ¼ that concentration (6.25/1). 120 animals were drawn from stock cultures and randomly distributed as follows: 20 were placed each in 20-ml beakers containing 10 ml of medium. 20 were placed together in a 100 mm stacking dish containing 200 ml of medium. 20 were placed each in 50-ml beakers containing 40 ml of medium. 20 were placed together in a 200 mm stacking dish containing 800 ml of medium. 20 were placed each in 400-ml beakers containing 160 ml of medium. 20 were placed together in a vessel 325 mm in diameter containing 3200 ml of medium. Posterior fission products were removed and recorded daily as in earlier experiments. The experiment continued for 53 days. The total numbers of fission products for each of the six groups is summarized in Table II. Note the lack of dependence between total numbers of fission products and the density at which the animals were maintained within both the isolated and social sets. Of the 20 isolates at the lowest density (6.25/1), 3 of the animals failed to fission, turned sexual, and deposited cocoons, all of which proved to be sterile. The 78 in parentheses
TABLE II
THE INDEPENDENCE BETWEEN DENSITY AND FISSION RATE FOR BOTH ISOLATED AND SOCIAL ANIMALS MAINTAINED AT THE THREE DENSITIES INDICATED
The figure in parentheses is the corrected number of fission products for the isolates maintained at 6.25/l, three of which turned sexual. See text for details.

| Density | Isolates | Group |
|---------|----------|-------|
| 100     | 79       | 29    |
| 25      | 82       | 23    |
| 6.25    | 66 (78)  | 27    |

in Table II is the corrected figure, assuming all 20 had produced fission products. Using this corrected figure, the isolates produced a total of 239 fission products and the social animals produced a total of 79 fission products, a ratio of 3.02/1 comparable to the ratios observed earlier in Figs. 5 and 6. The failure to demonstrate a density-dependent component can be taken as further evidence against a regulatory role for factors elaborated into the environment. The failure to demonstrate any effect of density on reproductive rate called for a re-examination of the effect of absolute population size on the numerical increase of the population.

Doubling Period as a Function of Absolute Population Size
In order to critically examine the kinetics of population growth, it was necessary to initiate the experiment with the animals all in phase. This was accomplished by isolating individuals from a prior social regime and then starting each population set with animals all of which had fissioned during the previous 24 h. Seven population sizes were established all at the same density of 25 animals/l. Each of the seven sets consisted of 24 animals distributed as follows: 24 isolates each in 40 ml; 12 pairs each in 80 ml; eight sets of 3 animals each in 120 ml; six sets of 4 each in 160 ml; four sets of 6 each in 240 ml; two sets of 12 each in 480 ml; one set of 24 animals in 960 ml. Posterior fission products were removed and recorded daily. A record was also kept of the fission histories of each of the 24 isolates. This information was subsequently used to evaluate the extent to which fission period was under genetic control as opposed to control by random factors. Observations were continued for all of the seven population sets until the slowest set had produced 24 fission products. This proved to be until day 40 when the single population of 24 animals in 960 ml produced the 24th fission product. Fig. 8
summarizes the findings. The ordinate is the total cumulative number of fission products and the abscissa is time in days. The lower dotted line intersects each trace at the 50% level of 12 fission products, while the upper dotted line intersects each trace at the 100% level of 24 fission products, which for the following treatment is defined as the doubling time. Note the sigmoid nature of the functions for population sizes of two or more animals. The pertinent numerical data read from Fig. 8 appear in Table III.

![Figure 8](image)

**Figure 8.** The total cumulative number of fission products (ordinate) as a function of time (abscissa) for seven population sizes each consisting of 24 animals. The experiment was initiated with animals all of which had fissioned during the previous 24 h. The numbers within the figure indicate the number of individuals in each population set or the number of animals in each vessel. The lower dotted line intersects each trace at the 50% level of 12 fission products. The upper dotted line intersects each trace at the 100% level of 24 fission products or the doubling time. Density is constant throughout at 25/1. See the text and Table III for the quantitative treatment of the data.

**TABLE III**

SUMMARY OF QUANTITATIVE DATA READ FROM FIG. 8

The model data was obtained from the frequency distribution of fission periods depicted in Fig. 11. See text for details.

| Population size | Model data Range | Mean | Actual data | Doubling time (days) | Total number of products (day 40) |
|-----------------|------------------|------|-------------|----------------------|----------------------------------|
| 1               | 10.2-11.3        | 10.7 | 11.2        | 15.4                 | 84                               |
| 2               | 13.0-15.8        | 14.9 | 15.0        | 23.0                 | 45                               |
| 3               | 15.0-19.1        | 17.2 | 16.0        | 26.0                 | 47                               |
| 4               | 16.5-20.8        | 19.0 | 17.8        | 32.0                 | 34                               |
| 6               | 17.8-24.3        | 21.2 | 23.6        | 34.0                 | 27                               |
| 12              | 21.0-31.0        | 25.0 | 21.3        | 36.0                 | 27                               |
| 24              | 23.0-37.0        | 29.0 | 31.0        | 40.0                 | 24                               |
Treatment of the Data

When the doubling time in days (ordinate) was plotted vs. the population size (abscissa) the points fell on what appeared to be a hyperbola of the general form:

\[ P_N = \frac{P_M \cdot N}{K + N}, \]

where \( P_N \) is the doubling time for a population of \( N \) individuals, \( P_M \) is the maximum doubling time approached by an infinitely large population, \( N \) is the number of individuals in each population or the number of animals in each dish, and \( K \) is the value of \( N \) at which \( P_N \) is one-half \( P_M \).

Note the identity to the Michaelis equation for enzyme kinetics, with the substitution of \( P_N \) for velocity, \( P_M \) for maximum velocity, and \( N \) for substrate concentration.

To estimate the constants in the expression, the reciprocal and linear form of the equation was used as is done in the treatment of enzyme data:

\[ \frac{1}{P_N} = \frac{1}{P_M} + \frac{K}{P_M} \cdot \left( \frac{1}{N} \right). \]

The reciprocal of period is plotted vs. the reciprocal of population size in Fig. 9. The straight line was fitted by the method of least squares. The equation of the line is as follows:

\[ \frac{1}{P_N} = 0.0231 + 0.0461 \cdot \left( \frac{1}{N} \right). \]

The coefficient of determination \( (R^2) \) for the line is 0.982. The reciprocal of period is, of course, frequency or fission rate. Note the dimensional identity.

![Figure 9](image-url)
of this equation with the expression previously developed to fit the data in Fig. 7. The reciprocal of the intercept value of 0.0231 is 43.3 days, which represents the calculated doubling period of an infinitely large population. \( K \) in the expression has the value 1.87, which is to say that a theoretical population consisting of 1.87 animals would have a doubling period equal to one-half of 43.3 days or 21.65 days.

**A Model System**

The following is an attempt at a model to explain the curious paradox that while population growth was independent of density, it was nevertheless somehow regulated by the absolute number of animals in the population.

The first question was "How reproducible is the fission period of a particular animal?" To answer this question, the fission histories of the 24 isolated individuals from the previous experiment were examined. Fig. 10 is a scatter plot of the fission period of an individual (ordinate) plotted vs. the next fission period of the same individual (abscissa). The straight line was fitted by the method of least squares.

![Figure 10](attachment:image.png)

**Figure 10.** A scatter plot of the relationship between the duration of a particular fission period (ordinate) plotted vs. the duration of the next fission period of the same animal (abscissa). The straight line was fitted by the method of least squares.

The equation of the line is as follows:

\[
(fission \ period)_N = 5.42 + 0.475 (fission \ period)_{N+1}.
\]

If fission period were determined by strictly genetic factors, one would anticipate a straight line with a slope of 1 passing through the origin. The coefficient of determination \( (R^2) \) is 0.313, indicating a low degree of correlation between successive fission periods. The positive slope suggests some genetic determination of fission period, yet a great deal of randomness is evident in the data. One source of the randomness may be the relative size of the posterior fragment from period to period. While no attempt was made to quantitate the size of the posterior fragment, inspection disclosed that the size of the posterior fragment was extremely variable. Thus, if an
animal happened to produce a large posterior fragment when fissioning, one would anticipate the next period to be of long duration simply because a relatively large fraction of the animal must be regenerated. Conversely, if the animal produced a small posterior fragment, the next period would be short because a small fraction of the animal must be regenerated before the next fission.

The second question was “What is the frequency distribution of fission period for a relatively large population of animals?” A total of 200 fission periods were determined by isolating individuals in 40 ml of medium and maintaining them until each had fissioned twice. The frequency distribution of the 200 periods appears in Fig. 11. The range is from 5 to 21 days, with a mean period of 10.68 days. These 200 periods form the basis for the following model.

Note in Fig. 8 that the maximum rate of increase (maximum slope) for the population sets tends to occur at the intersection of each trace with the 50% level indicated by the lower dotted line. The time at which the 50% level is reached is defined as the mean period ($P_n$) for each of the seven populations sets. The tendency toward synchronization of population growth suggested that the increase in numbers might be regulated by a pacemaker animal which would be the slowest animal in the population, or that animal with the longest fission period. The simple pacemaker model fails since the mean period of 31 days for the largest population (24 animals in 960 ml) far exceeds the longest period of 21 days for isolated individuals (Fig. 11). Accordingly, a modified pacemaker model was generated based on the following two assumptions.

First, each animal is assumed to resemble a relaxation oscillator, like a pipette washer, producing inhibitor for a period of time which varies from animal to animal, as well as from period to period. At the end of this variable period, the level of inhibitor falls and fission occurs. If however, the inhibitor
could be transmitted to another animal from the slowest animal in the population, the net effect would be to extend the period of the slow animal, and hence the entire population. The second assumption is that the ultimate sink for the inhibitor would be the fastest animal in the population, which one would assume to be that animal with the lowest concentration of endogenous inhibitor. The inhibitor is thus regarded as flowing directly from animal to animal through the population in accordance with the concentration gradient from the slowest to the fastest animal until the level of inhibitor reaches a maximum concentration when it subsequently falls, thus tending to synchronize the entire population. Evidence for synchronization of population growth is evident in Fig. 8 as well as in several earlier figures in this paper. The following empirical expression was developed to test the model hypothesis with actual data:

$$\bar{P}_N = P_L + (P_L - P_s),$$

where $\bar{P}_N$ is the mean period of the population of $N$ animals or the time to 50\% fissioning, $P_L$ is the period of the animal with the longest period in the population, and $P_s$ is the period of the animal with the shortest period in the population.

The 200 periods represented in Fig. 11 were each written on a reference card. The deck was repeatedly randomized, after which the cards were dealt out in linear array in groups of 24. The theoretical periods were then calculated, employing the above expression, by recording the periods in linear sequence for singles, groups of 2, 3, 4, 6, 12, and 24. For isolates, the second term in the expression is zero and the period is the mean of the 24 periods. The mean periods were calculated 10 times for each of the seven population sizes. The range and mean of the calculated periods appear with the actual mean periods in Table III. In every instance, the actual periods fall within the range of model periods obtained from the above expression. The model, in the interest of simplicity, neglects the distribution and values of the intermediate periods in each theoretical population. It is difficult to test the model hypothesis directly due to the great variability in the successive periods for individual animals (Fig. 10), but the agreement with the actual data indicates that the model is a reasonable one.

**DISCUSSION**

The regulatory mechanism proposed here depends on the assumption of an inhibitor. Lender and Zghal (6) have correlated the fission cycle in *D. gonocephala* with the number of neurosecretory cells in the brain. At 21°C *D. gonocephala* has a fission cycle of approximately 21 days. The number of neurosecretory cells drops precipitously from about 90 on day 19 of the cycle to about 30 on day 21 when fission occurs. The number of neurosecretory
cells continues to drop to zero on day 5 after which the numbers increase again to a maximum on day 19. The number of neurosecretory cells thus oscillates with a period coincident with the fission cycle. Lender (5) has also shown that the numbers of neurosecretory cells in the brain remains high during the sexual state when fissioning is inhibited. Morita and Best (7) have examined the ultrastructure of the neurosecretory system and found that neurosecretory material is released from vesicles in which it is stored and travels down the axons toward the posterior of the animal. It is possible that the neurosecretion, or a product derived from it, is the inhibitory factor which is the essential feature of the model system developed here.

There are two major arguments against the view that contact induces a qualitative change in the animals involved. First and most important, the fission periods of isolated individuals, not subject to contact, fit the kinetic expression relating fission period to population size, indicating that no new mechanism is initiated in response to contact. Second, it is difficult to account for the synchronization of fissioning by the assumption of a unique mechanism dependent on contact for its release. The tendency of the population to behave as a superorganism can be most simply explained by the assumption that all of the animals in the population share the same primary regulatory factor.

Best, Goodman, and Pigon (1) conclude that the suppression of fissioning by the brain is contingent on population density, a reasonable conclusion in view of their experimental design. The experiments reported here, which distinguish between density and absolute number, fail to support that conclusion. The model system depends only on the range in variation of fission period within the population. The range of fission periods is, in turn, dependent simply on the total number of individuals comprising the population structure. Thus it is unnecessary to invoke new forces unique to the social regime. Future experiments are designed to isolate and characterize the factor or factors which mediate this unusual regulatory mechanism.

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