MORPHOGENESIS AS INFLUENCED BY LOCALLY ADMINISTERED MAGNETIC FIELDS

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ABSTRACT An alteration in morphogenetic development induced in *Drosophila melanogaster* pupae by exposure to a magnetic probe was shown to persist for more than 30 generations. With succeeding inbred crossings, an initial increase in the time of development through the embryonic and postembryonic stages gradually approached the level found in control cultures. As the development time decreased, a concomitant increase occurred in the yield of progeny. The pattern of morphogenesis suggested a condition of homeostasis operating in an oscillating epigenetic system. The concept of a feedback control mechanism was employed to examine details of the alterations in development time. The data were compatible with this model and rates of recovery from an initial perturbation were determined in several series inbred for a large number of generations. Variations in rate constants and mechanisms involved in the magnetic field inhibition are discussed.

INTRODUCTION

A previous study disclosed that exposure of *Drosophila melanogaster* pupae to a magnetic probe with a high flux gradient produced a retardation in the embryonic and postembryonic stages of development (1). Even though only those pupae producing first generation flies were exposed to the probe, the developmental retardation was genetically transferred to succeeding inbred generations. Principally, the alterations in embryogenesis were expressed through probe-treated male pupae.

At the time this work was reported, transference of the increase in development time had been followed through several series consisting of 10–12 inbred generations of flies; however, it was not known how long the effect would persist. Specific characteristics within the patterns of development were also to be determined. Results are presented here which show that a lengthened development time may be carried through 30 generations of probe-treated flies. It is also demonstrated that there is a concomitant change in the yield of progeny as the development time approaches the level found in control stocks.

The present findings, in general, are in accord with an enzyme inhibition theory proposed in the initial paper (1). As an extension of this hypothesis, the probe-induced alterations are considered in relation to a condition of homeostasis operating

over a number of generations. The occurrence of an oscillating mechanism in a perturbed epigenetic system was suggested in the specific patterns of development. By utilizing the concept of a feedback control mechanism the rate of recovery from the probe-induced perturbation was determined in those series inbred for a large number of generations.

Recently, it was reported by Close and Beischer (2) that both homogeneous and inhomogeneous magnetic fields have little or no influence on *Drosophila*. There are, however, important differences between the investigations of Close and Beischer and those described in the following sections. The most significant experimental variations appear to be related to the type and length of exposure. These workers used whole body exposure over the entire life cycle, whereas the probe is applied in a localized region and for a comparatively short duration.

METHODS

The probe coil was wound with No. 34 insulated copper wire. The core was a 6 mm diameter rod of low remnant field. A 1 inch section at one end of the core was prepared with a conical taper down to a final diameter of 0.125 mm with the tip machined flat. A model 110 Bell gauss meter with a sensor area of approximately 1×2 mm was used to map the field characteristics of the probe, (ratio of meter sensor area to the probe tip area approximately 160:1). In Table I are listed the measured field strength and gradient along with the design characteristics of the probe.

The probe was mounted in a vertical position over a horizontal glass microscope slide which in turn was fastened to a rack and pinion to provide precise vertical adjustment. With the specimen on the glass slide the table could be brought in proximity of the magnetic probe tip; approximately a 0.5 mm air gap was provided between the probe and test specimen. The details of treating the flies, the procedure of counting, the type of medium, laboratory conditions, etc., were as previously reported (1).

Development time as discussed here was taken as the number of days from the start of a new culture until eclosion. Unless otherwise specified, the flies were wild type Ore-R strain. It was previously determined that the mean development time in control cultures is 13.6 ± 1.12 days. Although this is somewhat longer than the usual value reported in the literature, it was shown that it is characteristic of this strain and is not due to overcrowding or larval competition (1). This 13.6 day value also includes approximately a 24 hr period during which the flies recover from etherization when transferred to a new culture. The development time was considered as being significantly altered if the value was greater than 16 days, the upper extreme found in control series. Throughout the series reported here control cultures were grown concurrently with the probe-treated groups. Over 150 separate control cultures were examined and the development time remained at the previously reported 13.6 day value. The individual data points for the control cultures were not plotted with the test data; however, the mean and standard errors of the controls are indicated on the graphical presentations.

RESULTS

In the original investigation, it was found that the pupal stage of development appeared to be very sensitive to the magnetic probe exposure. For this reason, in the work reported here, emphasis has been placed on probe effects in pupae. The egg and larval stages will be more closely examined in later studies.
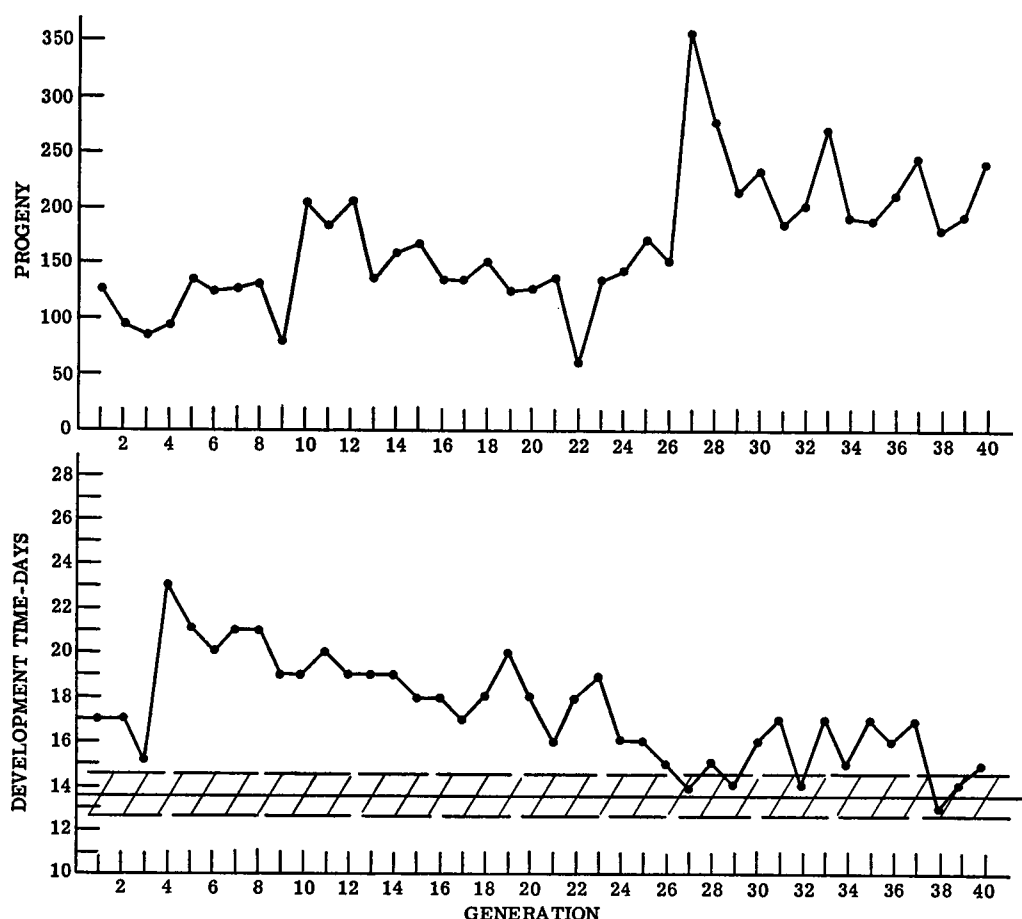
FIGURE 1 Individual generation values of progeny and development time from males re-
sulting from probe-treated P_1 pupae, crossed with normal females (pupae 21 hr age, 10 min
exposure using an S orientation). The mean and standard error for the development time
in over 150 control cultures are indicated by the solid horizontal line and the cross-hatched
region.

Development Time Alterations in Inbred Cultures

Five different series of inbred generations originating from probe-treated pupae
have been continued beyond the F_{30} generation. In all of these series, it was apparent
after about 15 generations that the development times were slowly decreasing.
With the gradual reduction in the development time, there was also a concomitant
increase in the progeny yields. The inverse trends in the development time and
progeny curves are shown in Fig. 1 for P_1 pupae treated 10 min at 21 hr age using
an S orientation (treated males crossed with normal females; see Fig. 3 in reference
1). The points in Fig. 1 represent the progeny and development time values for

individual generations. In generation 27 the development time was in the normal range (14 days); however, the development time did not thereafter remain at the normal level. (Horizontal line and cross-hatched region in Fig. 1 indicate the mean and standard error for control cultures.) A comparison of the peaks and valleys in Fig. 1 certainly does not indicate a one to one correspondence between the two curves. After generation 26, the progeny level is considerably higher than the established mean for controls (150 ± 32) (1). It is anticipated that the progeny level will eventually return to the normal range as the development alteration continues to decrease; this point will be examined in future studies.

In various series, which have demonstrated development time alterations through male treated pupae, exposures were also given female pupae of a corresponding age. It was found that regardless of the age of the female pupae there was no alteration in the development time when crossed with untreated males. After treatment of several hundred pupae at various ages, there has been only one exception to the effect being carried exclusively through male progenitors. This exception occurred in a female pupa probe-treated 10 min at 98 ± 1 hr age using an N orientation. In Fig. 2 is shown the development time alteration and progeny from this single female crossed with normal males. (Horizontal line and cross-hatched area again indicate mean and standard error controls.) A cyclic pattern in the development time curve is indicated; in fact, the general shape of the curve is suggestive of a system with an exponential decay. (The implications of this are discussed in a later section.)

Influence of Exposure in Head Region

Many of the probe-treated pupae that survived exposure in the head region disclosed the development time alteration. The pattern of this alteration was essentially the same as for those pupae exposed in the gonadal region. In Fig. 3 are two series from head-exposed pupae (40 hr old) demonstrating variations in progeny and development time; the points on the solid curve represent individual generations from an initial culture containing both probe-treated males and females and the broken line is a series from treated males crossed with normal females.

The groups shown in Fig. 3 were intentionally mated on different dates so that the patterns could be examined in nontemporally corresponding series. Although the laboratory conditions of diet, temperature, etc., were held constant, "external" environmental factors such as cosmic rays and solar flares may influence the yield of progeny (3). In spite of different external environmental influences, the curves in Fig. 3 are similar in appearance. The gradual rise in progeny level with decreasing development time is also apparent in these head-treated series.

Both of the development time curves in Fig. 3 indicate a cyclic pattern similar to that shown in Fig. 2 for the treated female pupa. The occurrence of the gradual decrease in development time and the increase in progeny suggests the presence of a condition of homeostasis operating in these systems. There appears to be a return to an equilibrium condition in a system initially perturbed by the magnetic probe.
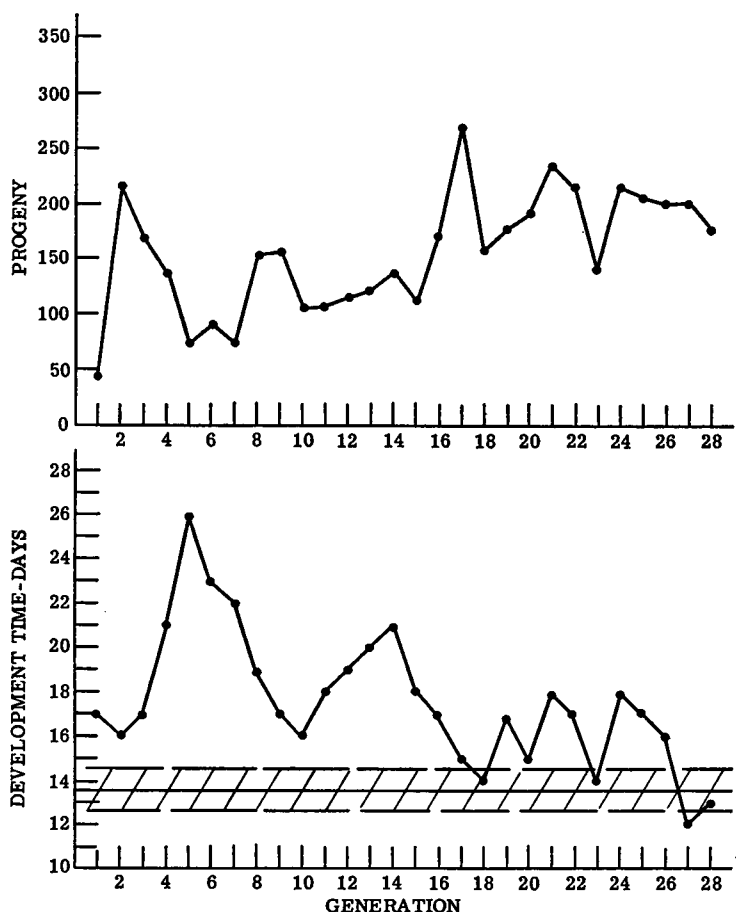
FIGURE 2 An exceptional female demonstrating alterations in development time and progeny after crossing with normal males. Pupa 98 ± 1 hr age, exposed 10 min using an N orientation. The mean and standard error for the development time in over 150 control cultures are indicated by the solid horizontal line and the cross-hatched region

This return to the original equilibrium is indicated by the fact that in spite of the cyclic pattern in the development time the values gradually approach the control level designated by the cross-hatched region at the bottom of the figure.

Perturbations in an Epigenetic System

A theory was previously advanced that the magnetic probe introduces a perturbation in a developmental enzyme system (1). The magnetic field induced inhibition was expressed by the general equilibrium equation:

$$E + I_m \underset{k_2}{\overset{k_1}{\rightleftharpoons}} E_m \tag{1}$$

where E is the initial equilibrium concentration of the enzyme, I_m is the induced perturbation of the magnetic field, and E_m, the final concentration of the altered enzyme system. The reaction rate k_1 represents the probe-induced alteration and k_2, the rate of return of the altered enzyme to the original activity.
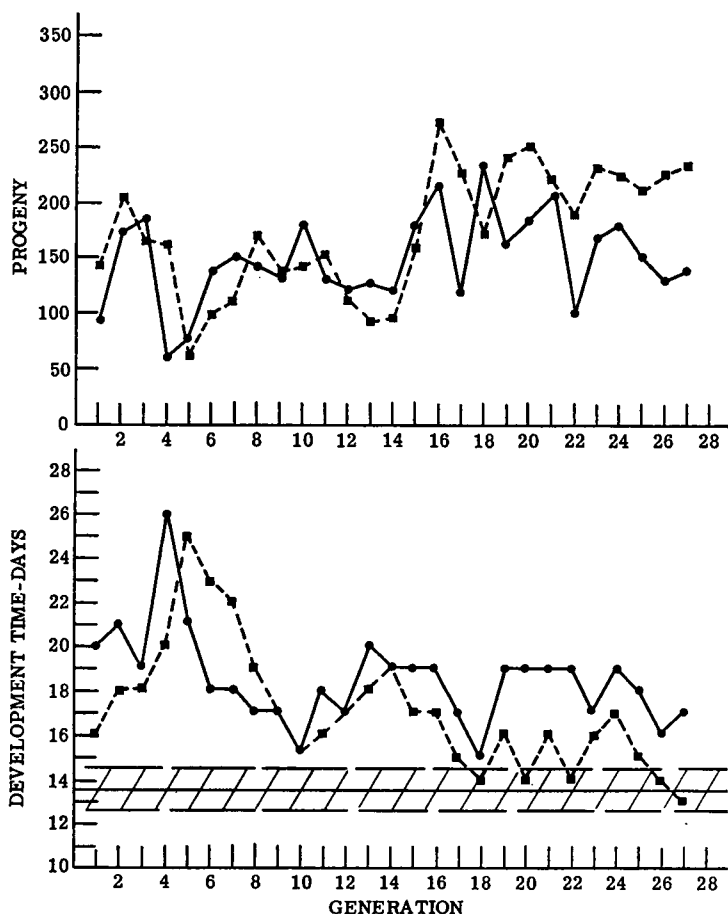

FIGURE 3   Alteration of development time and progeny in flies resulting from head exposed pupae. Solid line indicates probe exposure of both sexes; broken line, treated males crossed with normal females. Pupae 40 hr old and exposed 10 min using an N orientation. The mean and standard error for the development time in over 150 control cultures are indicated by the solid horizontal line and the cross-hatched region.

Since the developmental alteration is transferred to succeeding generations of flies, the rate of restoration k_2 to the original enzyme activity was assumed to be negligible and irreversible inhibition was considered. The offspring or progeny yields were postulated as being an expression of the final end product from an altered developmental enzyme E_m and the development time as being related to the

k_1 reaction rate. By examining mean values taken from the first four inbred generations of flies, it was shown that the experimental data were in accord with the first order reaction kinetics.

Based on these kinetic variations, the probe-induced alterations in development are postulated as taking place in an epigenetic system in which the major activities are considered to be the biosynthesis, diffusion, and interaction of molecules and macromolecules. The requirements for the feedback process in the epigenetic control mechanisms are, in the case of the probe-treated series, transmitted to succeeding generations. With regard to an hypothesis concerning the inheritance from individual probe-treated flies to the following generations, we must first consider two previously reported (1) experimental observations: first, the inheritance of an altered development time appears to be non-Mendelian and second, this alteration occurs primarily through treated males. These factors tend to indicate cytoplasmic involvement. One likely site for magnetically induced energy changes is within the ordered structure of the mitochondria. A slight reorientation of intermitochondrial groups such as ATP could influence the rate of oxidative phosphorylation. Through cytoplasmic inheritance, the mitochondrial system (or some other organelle) with its altered reaction rate is transferred to the next generation. Such a perturbed cytoplasmic system would for this type of ontogenetic inheritance require a very long relaxation time. Since the alteration in the cytoplasmic system is, on the basis of single generations, essentially an irreversible reaction, this indicates, as pointed out by Goodwin (4), that an induced oscillation in the system would not be inconsistent with thermodynamic laws. The specific characteristics of the development time curves (particularly Fig. 2) suggest the presence of such an oscillating mechanism.

The increase in progeny as the development time approaches the control level may be an "overshoot" mechanism in the homeostasis condition resulting from an initial response to the magnetic field alteration. In terms of population levels this situation has been discussed by Prosser (5) when considering the effects of environmental stresses such as temperature shocks. Examination of metabolic rate processes discloses that there is a tendency of many systems to overshoot with a new stabilized level brought about by the environmental stress. Feedback mechanisms operating through enzyme systems tend to restore the organisms to normal.

The tendency of a perturbed system to overshoot and undershoot the equilibrium reaction rate could lead to a condition of oscillation which would damp out with time. In the case of the development time alteration, the relaxation time is related to the rate at which the system approaches the steady-state level following the disturbance. The influence of external, uncontrollable environmental parameters which might perturb individual generations would be considered as having by comparison a small relaxation time and would introduce only minor alterations or "noise" in the much slower system (large relaxation time) altered by the magnetic probe.

Based on these considerations, the data were examined in relation to the operation of an oscillating feedback mechanism. The advantage of exploring this type of model

lies in the fact that even though the enzyme or enzymes involved in the interaction with the magnetic field are not as yet known specifically, we may by examining the kinetics of the interacting species learn something of the mechanisms involved in the temporal annulment of the intercellular perturbations. Following the method of Goodwin (4) we will allow the distribution function ρ_0 to represent the equilibrium condition in the perturbed molecular or enzyme system after removal of the magnetic probe. In terms of ontogenesis ρ_0 represents a new equilibrium distribution within a cytoplasmic organelle. As postulated, this magnetically altered molecular distribution is capable of being genetically transferred. This new distribution function differs from the original molecular equilibrium function ρ by a small quantity Δ_ρ written as

$$\rho = \rho_0 + \Delta_\rho . \tag{2}$$

For a small disturbance in a dynamic system the rate $(-d\Delta_\rho/dt)$ at which the effect is annulled follows first order kinetics (6), that is, the rate is proportional to the magnitude of the perturbation or concentration of the altered molecular species; therefore,

$$-d\Delta_\rho/dt = k\Delta_\rho \tag{3}$$

where k is the rate constant. After performing the integration, this gives the relation

$$\Delta_\rho = (\Delta_\rho)_0 e^{-kt}. \tag{4}$$

The unit of time t in equation 4 is in terms of sequential generations; thus, with increasing generation number, one would expect an exponential decrease in the disturbance. Since the rate of restoration k_2 in equation 1 is taken as 0 for a given generation, the development time T will be considered as being proportional to the molecular distribution as

$$T = K\rho \tag{5}$$

where K is a proportionality constant. One method of comparing the exponential form of equation 4 with the experimental data is to examine the envelope connecting the points of maximum amplitude in an oscillating system such as suggested in the Fig. 2 data. The maximum points are not as clearly defined in all the development time curves as, for example, in Fig. 1 and for this reason, it appeared more advantageous to examine mean values within a given period of oscillation. Assuming each perturbed system has a constant period of oscillation, an examination of Figs. 2 and 3 reveals that the maximum points in the development time curves occur roughly within periods of seven to nine generations; consequently, for calculating mean values, the constant period was taken as being eight generations. From equation 5
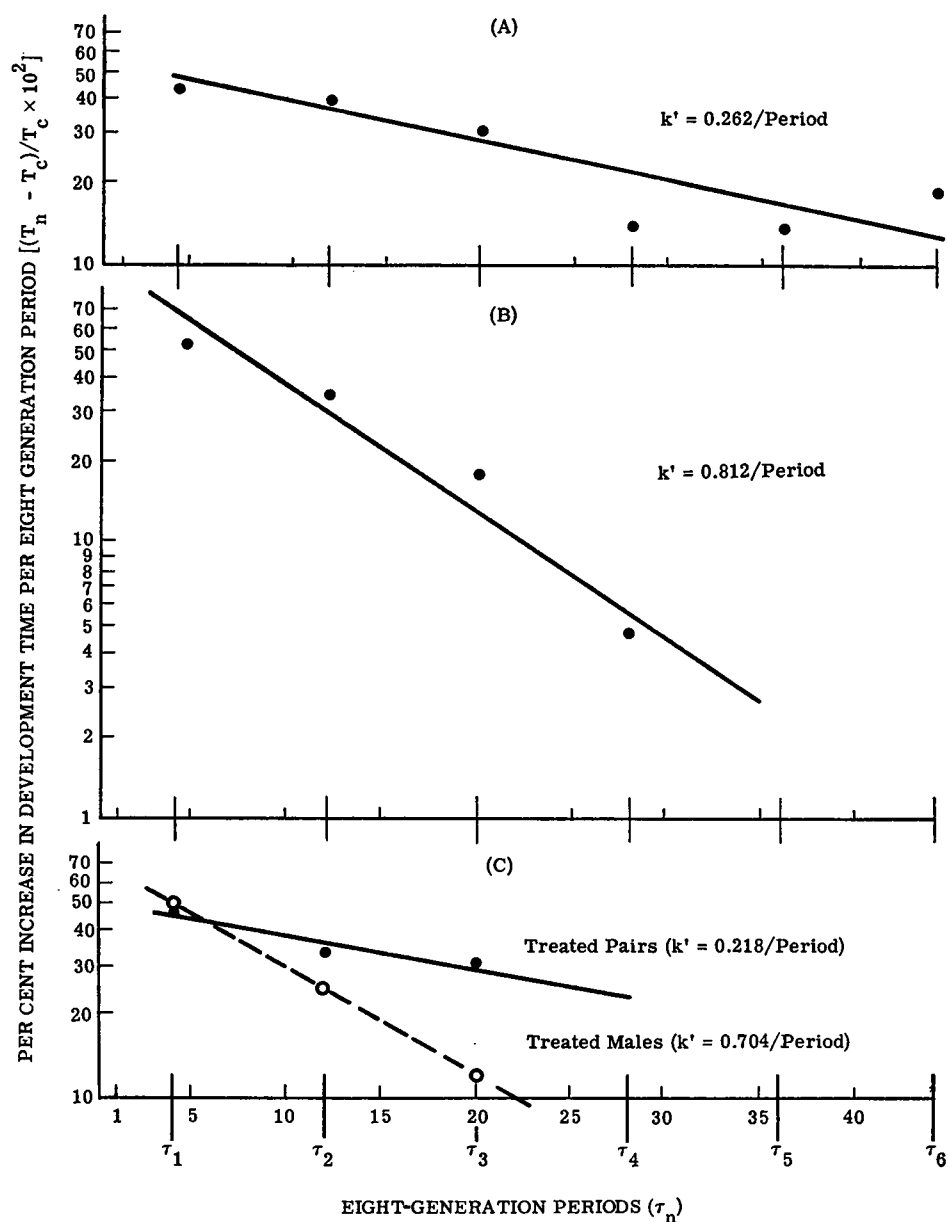
FIGURE 4 Arrangement of development time data into eight generation periods; k' is the rate of recovery from the probe perturbation. Curves A, B, and C are the data taken from Figs. 1, 2, and 3 respectively.

and the exponential form of equation 4 the per cent increase in development time may be expressed in terms of oscillating periods

$$\ln\left(\frac{T_n - T_c}{T_c} \times 10^2\right) = -k'\tau_n \tag{6}$$

where T_n is the mean development time (or average perturbation) in an eight generation period $\tau_1, \tau_2 \cdots \tau_n$, T_c is the development time in control cultures (13.6 days), and k' is a constant which expresses the rate at which the induced alteration decreases per eight generation period of oscillation. The reciprocal of this rate constant may be taken as a measure of the relaxation time in the perturbed system.

The data from Figs. 1, 2, and 3 were arranged in eight-generation periods and plotted in Fig. 4 according to the equation 6 relationship. In general, it may be seen that the curves are approximately linear as predicted by the hypothesis of oscillations

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TABLE I

DESIGN AND FIELD CHARACTERISTICS OF THE MAGNETIC PROBE

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Diameter of probe tip	0.125 mm
Measured field strength at tip	150 gauss
Measured field gradient in first 2 mm from probe tip	55 gauss/mm
Applied voltage	22.5 v
Approximate DC current	12 ma
"effective field strength", calculated from ratio of meter sensor area to probe tip area (see text)	$\sim24 \times 10^3$ gauss

in perturbed systems. The specific rate constant for each group is also given in Fig. 4 and the variations between the inbred series are of interest. For example, the probe-treated males crossed with normal females (curve A) disclosed a lower rate of return or larger relaxation time than the series (curve B) from a treated female crossed with normal males. This rate difference in the series exposed in the gonadal regions is in accord with the effect being expressed primarily through males. In the case of the head-treated series the females appear to have a more pronounced influence on the rate constant. For example, when both sexes were probe treated, the rate (C, solid line) of return was much slower (larger relaxation time) than when only males were exposed (C, dashed line).

DISCUSSION

Perhaps a final comment should be made concerning the fact that the probe treatment consistently induces an increase in the development time rather than a decrease. This is in agreement with the hypothesis of an enzymatic inhibition in a perturbed system. It has been pointed out (4) that a perturbation occurring in an asymmetrical feedback system such as alterations in protein or enzyme concentrations would lead

to a slowing down of the rhythmic processes in the organism. In this case, the biological "clock" being influenced by the magnetic field is one operating over the entire period of morphogenesis (13.6 days), with much slower periods than in fluctuations usually associated with diurnal rhythms. By incorporating probe exposures with biochemical analyses, it should be possible to elucidate the degree of perturbation in specific enzyme systems.

The kinetics of enzyme inhibition involve adsorption and diffusion mechanisms. An hypothesis advanced by Liboff (7) suggests that the inhibiting influence of magnetic fields in cell development is based on alterations in the diffusion of intercellular charged particles. This author calculated that perturbations should occur using fields in the order of 10^5 gauss and this is of the same order of magnitude as the calculated value of the "effective field strength" at the tip of the magnetic probe (Table I). It was also pointed out that for maximum diffusional effects the flow of charged particles should be parallel to the direction of the field. In the case of cells in vivo one might expect a random orientation of nuclei as well as other intercellular particles. Perhaps there are sufficient organelles such as mitochondria which fortuitously lie at the optimum orientation for diffusional effects to alter the reaction kinetics. One might also speculate that in the nonuniform field the intercellular particles become oriented so that diffusional effects are enhanced. These speculations could be examined by cytological observations in the presence of the probe field.

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