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Nabil M. Amer

December 1965

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THE EFFECTS OF HOMOGENEOUS MAGNETIC FIELDS, AMBIENT GAS COMPOSITION AND TEMPERATURE ON DEVELOPMENT OF TRIBOLIUM CONFUSUM

Nabil M. Amer
(Ph. D. thesis)
December 1965

The Effects of Homogeneous Magnetic Fields, Ambient Gas Composition and Temperature On Development of Tribolium confusum

Nabil M. Amer
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December 1965

Abstract

We were able to establish the existence of interaction between homogeneous magnetic fields and the kinetics of development and differentiation of the pupa of Tribolium. This interaction leads to alteration in the normal developmental path. The degree and type of alteration are functions of the combination of physical and chemical parameters applied to the system. Essentially, magnetic fields have two basic effects: one is equivalent to cooling, and the other is the enhancement of the interaction with ambient oxygen, possibly through influencing the rates of oxidation. The data we obtained from Tribolium as well as more recent preliminary findings with liquid crystals of cholesteric compounds substantiate this hypothesis.

Out on't! I've searched philosophy,
Medicine and Law I've sifted in vain,
And (God above!) to Theology
I've given the best of heart and brain.
Pitiful fool! I ponder and pore
And grow no wiser than before.
Ah, but I wear the master's gown,
The doctor's scarlet; up and down
These ten years past with cons and pros
I've led my scholars by the nose.

—Johann Wolfgang von Goethe
from *Faust*

I. INTRODUCTION

A. General Problem.

It is plausible to assume that the living state is the resultant of an intricate interaction between the basic elements making up the living system and known physical forces of the universe. Since magnetic fields constitute a cornerstone in the physical theory, it is then logical to test for the effects of such fields upon biological phenomena.

As early as the 17th century, the influence of magnetic fields on living systems was investigated. Johann Baptista van Helmont wrote about animal magnetism which earned him the condemnation of the Holy Office. Although the literature in such a realm is not meager, the reproducibility of the claimed results is vulnerable to challenge. Recently, a survey of the literature of the biological effects of magnetic fields was published by the Federation of American Societies for Experimental Biology.(1) This reference is not limited to the effects of magnetic fields upon biological systems; for it covers areas of research such as microwave spectroscopy of free radicals, nuclear magnetic resonance and susceptibility studies of biological systems. In 1963, Barnothy(2) edited a book aimed at surveying the current research in the field of "Biomagnetism". The majority, if not all of the experimental reserach published thus far, although diverse and sometimes contradictory, has one common characteristic, and that is the lack of a plausible hypothesis underlying the execution of the experiments.

If magnetic fields were to influence biological systems, those systems have to contain "centers" whose properties are such that they would interact with the applied field. Two possibilities offer themselves; one is the assumption that such centers would be of a paramagnetic nature. This assumption is supported by experimental evidence.(3,4,5,6,7,8,9,10,11) The other alternative is that the system contains electric current propagating with respect to an external fixed magnetic field thus leading to the induction of voltage gradient.

The initial purpose of this study was to investigate the effect of magnetic fields upon the behavior of either naturally occuring or induced free radicals in a suitabe biological system. Within the context of free radicals and magnetic fields, oxygen has unique value. On one hand, the combination of molecular oxygen with radiation-induced radicals is of prime biological importance.(12,13,14,15) On the other, oxygen per se is paramagnetic. This two-fold nature led to the branching of this work with the aim of investigating the role of magnetic fields upon the combination of free radicals and oxygen.

The experimental biological system used in the work reported below was the pupae of Tribolium confusum.

B. Physical Factors.

Three physical factors constituted the principal variables in this work; these parameters are: 1. Homogeneous magnetic fields, 2. Thermal energy, 3. Ionizing radiation.

Magnetic Fields:

Although it is not of direct concern to us to trace the history of magnetism, a brief summation of such history may serve to set the stage in attampting to build a logical background to this work.

It is said that man's first encounterment with magnetic fields was the discovery of lodestone by a Cretan shepherd who was strongly drawn to the earth by his iron-tacked sandals while roaming about in Magnesia, a district in Thessaly where Magnetite, Fe_3O_4, is abundant. However, Bhatnagar and Mathur(16) argue that the first description of lodestone was that in the Vedas, the most ancient book of the Hindus. There is a vague reference in ancient Chinese literature to the "south-pointing chariots". Greeks and Romans had knowledge of some empirical facts and speculations about magnetic phenomena. Plato compared magnetism to poetic inspiration. Thales of Miletus (c. 600 B.C.) is said to have known the abnormal properties of magnets then known as "magnes". Later Lucretius, in De Natura Rerum, records repulsion: "Sometimes too it happens that the nature of iron is repelled from this stone, being in the habit of flying from and following it in turns."

The first artificial permanent magnets were iron needles "touched" by a lodestone. That must have taken place around 1200 A.D., where the French troubadour Guyot de Provins records in satirical poem "La Bible" a compass made of such a needle supported by floating straw.

Petrus Peregrinus of Maricourt in his "Epistola ad Sygerum de Foucancourt Miletem de Magnete," published in 1269, undertook the first systematic attempt to clarify the mystery that surrounded magnets. He was the first to introduce the term "magnetic poles" which he deduced experimentally. To him also is attributed the discovery that a piece of metal broken apart from a magnet also behaves like a magnet and that unlike poles attract each other while like ones repel.

However, to William Gilbert of Colchester (1540-1603) goes the credit for making magnetism a refined field of scientific endeavor by sifting the facts from the fiction. His classic masterpiece "De Magnete Magneticisque Corporibus et de Magno Magnete Tellure Physiologia Nova," published in 1600, is a delight to read for it set a meticulous example of fine experimental physics. Gilbert, himself a physician in the court of Queen Elizabeth, helped in differentiating between the magnetic and the "medicinal" properties of the lodestone; for in man's endless search to conquer diseases, magnets with all the mysterious and occult virtues that enveloped them were thought to be of beneficial medical value. Perhaps as a testimony to the exemplary scientific approach of Gilbert's work we cite two notable conclusions of his: 1. The earth itself behaves like a magnet, and, 2. Iron ceases to be attracted when red hot.

Descartes, to establish his physical system, had to involve himself in the controversial phenomenon of magnetism. Above all, he set to destroy any metaphysical or vitalistic interpretation of magnetism. To fit such phenommenon in a suitable slot of his cosmological system, he explained the attractive forces of a magnet are due to air rushing into magnets, thus thrusting magnets and iron together; he accounted for differences in the magnetic properties of iron and steel by the difference in their "pore" structure. It was Robert Boyle who, experimentally, disproved the pore hypothesis by placing a magnetic needle in a vessel connected to a vacuum pump; he found that the response of the needle to a magnet outside the vessel was the same whether the vessel was evacuated or not. Huygens substituted the air in Descartes' theory by the "ether" which "could exist even in vacuum." Oddly enough, Newton had very little to say about magnetism.

John Mitchell (1724-1793) and John Robison (1739-1805) conducted the earliest recorded quantitative experiments which led to their fundamental observation that "the attraction and repulsion power of magnets decreases as the squares of the distances from the respective poles increase."

Coulomb and Poisson utilized mathematical analysis to solve the physicochemical problems of magnetic phenomena. Poisson adopted the famous "Two fluid" theory in his analysis, according to which all magnetic substances consist of a large number of small magnetic particles containing equal quantities of positive and negative magnetic fluids. Later, this idea had to be abandoned in favor of Ampère's molecular current theory which does incorporate Oersted's accidental observation concerning the relationship between electricity and magnetism, and also the subsequent findings af Arago, Biot, Savart and Ampère, revealing that an electric current is invariably accompanied by a magnetic field. Faraday, who was lured into this field by these discoveries, introduced the law of electromagnetic induction given in the differential form by $\vec{\nabla} \times \vec{E} = -\frac{1}{c}\frac{\partial \vec{B}}{\partial t}$, thus establishing a reciprocal relationship between magnetism and electric currents which served as a supporting evidence in favor of Ampère's theory.

Faraday, in his thorough manner, extended the earlier findings of Brugmans, who observed the paramagnetic nature of cobalt and the diamagnatic properties of bismuth and antimony, by investigating a large number of substances in the solid, liquid and gaseous state for their magnetic properties. He made the conclusion that all matter, whether compounds or elements, possessed either diamagnetic (a term he first introduced) or "magnetic" property. Faraday introduced the concept of magnetic fields and that led him to test their influence on light; that investigation culminated in the discovery of the Faraday effect: the rotation of plane-polarized light upon passing through a medium in a direction parallel to magnetization. By then enough experimental observations were available to permit attempts to formulate a general theory of magnetism.

Weber in 1845 introduced a molecular concept to account for magnetism. He assumed that molecules in iron and steel are themselves permanent magnets capable of turning around their centers. In an unmagnetized piece of iron or steel these molecules are arranged randomly thus cancelling each other's external field; whereas in a magnetized piece of iron these molecules are ordered in a given direction, that of the magnetizing force. Weber's theory failed to account for two facts: 1. It does not explain the phenomenon of "residual" magnetism, and 2. It does not account for the internal resistance of the iron or steel which prevents the full alignment of all molecules. Ewing in 1890, after his work on the phenomenon of hysteresis, introduced the idea of sub-magnetization; i.e., when a piece of iron is unmagnetized, the molecules are arranged in groups, each group forming a stable magnetic unit with no external field. When an external magnetic field is applied, the magnetic induction lags behind the magnetizing forces. Later, Ewing abandoned the idea of magnetic molecules in favor of the view that revolving electrons within the atom give rise to magnetic properties; hence, conforming with the electronic theory of matter.

As mentioned above, it was Faraday who established the "pan-magnetism" of matter; be it paramagnetism or diamagnetism. However, a quantitative basis for this finding had to await Curie's work on the relationship between magnetic properties and temperature, Honda's invertigations with elements, and P. Pascal's detailed work on the influence of chemical combination, particularly of organic liquids on magnetic properties. These constituted the pillars on which the modern theory of magnetism was raised. This modern theory is based on the classical theory of Langevin. Later, and after the flourishing of the Quantum Theory, this theory had to be modified. An excellent approach to the modern theory of magnetism is found in Mattis' book.(17) We shall limit our discussion to diamagnetism and paramagnetism.

All types of matter possess some magnetic properties; in general these properties are: diamagnetism, paramagnetism and ferromagnetism. In a diamagnetic substance, when a magnetic field is being established, its time rate of change induces electromotive forces in the atoms, and these electromotive forces build up circulating currents inside the atoms. Due to the absence of resistence on the atomic scale, these currents persist as long as the magnetic field continues. The circulating currents result in magnetic moments, which are proportional to the applied field. Such moments are the cause of diamagnetic behavior. From Lenz's law, these moments oppose the external field. If the currents were to circulate not only within individual atoms, but a [illegible] rather large molecule, e.g., a benzene molecule, they circulate around the ring, i.e., with a large radius of the electronic current, leading to high susceptibility since susceptibility χ is proportional to the square of the [illegible] current radius. In metals, in the process of building up a magnetic field we induce large scale currents. These are the eddy currents which die down because of the electrical resistance. Hence they do not result in diamagnetism. The behavior of diamagnetic substances in external fields is explained according to Larmor's theorem which states that the superimposed field essentially leaves the form of the electronic orbits (within atoms or molecules) and their inclination to the magnetic lines of forces, as well as the motion in the orbits, unchanged, and merely leads to the superposition of a common precession of the orbit about the direction of the lines of force. Therefore, the motion of the system is the same as it would be in the absence of the field except for a superimposed uniform rotation around an axis parallel to the field.

An atom generally has a magnetic moment resulting from two contributions:

1. The orbital magnetic moment, arising from the circulation of the electrons around the nucleus, a phenomenon comparable to closed current loops, and
2. The spin magnetic moment, which is an inherent property of the electron.

In the absence of external magnetic fields, these can assume any arbitrary direction in space. In the presence of a field and according to quantum mechanical laws, we have space quantization, i.e., there are certain allowed states of orientation. These states are characterized by angular momenta spaced along the axis by integral or half integral multiple of $h/2\pi$. Since the magnetic moment is proportional to the angular momentum, thus we have definite components of magnetic moment along the axis resulting in definite energy levels, as the magnetic energy in the presence of the field equals the product of field strength and the component of magnetic moment along that field. At high temperature, all orientations would have equal likelihood of occuring; but at lower temperatures, the Boltzman factor, giving the probability of finding an atom with energy E is proportional to $e^{-E/kT}$, indicates that those orientations with low energy, or with the magnetic moment oriented along the field, are more likely than those with high energy, or with opposite orientation. This argument leads to an average magnetic moment proportional to the magnetic field strength and inversely proportional to the absolute temperature. This is the origin of paramagnetic effects. Atomic paramagnetism is closely related to the Zeeman effect, a phenomenon discovered in 1896 by Zeeman when he showed that the spectral lines could be decomposed into sets of lines, multiplets, if radiating atoms were subjected to intense magnetic fields. Zeeman's teacher, the Dutch Hendrick Antoon Lorentz explained, classically, this unorthodox finding utilizing the electron theory. A simple quantum mechanical explanation of this effect is given as follows: in relating magnetic moments to angular momenta in the presence of a field we find that the magnetic moment is equal to e/2mc times the angular momentum in the Gaussian system, where e is the electronic charge, m its mass and c is the velocity of light. As we mentioned above, angular moments are quantized in units of $h/2\pi$, and the natural unit of magnetic moment, $eh/4\pi mc$, called the Bohr magneton, is denoted by $\mu_B (= 9.2732 \times 10^{-21}$ erg/G). In a magnetic field B along the axis, the energy of the magnetic moment will be $-m\mu_B B$, the product of magnetic moment points along the field. Thus there will be a modification of the energy levels, produced by the external field, and proportional to it; this accounts for the Zeeman effect.

Paramagnetism has two interesting characteristics: temperature dependence and resonance effect. As we mentioned earlier, magnetization in paramagnetic substances results from the orientation of those atoms possessing permanent magnetic moment in applied magnetic fields; thermal agitation resists this orientation. Langevin's equation relates, classically, susceptibility to temperature as follows:

$$\chi = \frac{M}{H} = \frac{N\mu^2}{3kT} = \frac{c}{T}$$

The 1/T temperature dependence is known as Curie Law.

Earlier we showed that a magnetic atom in an external magnetic field has its axis precess around the field, equal or closely related to the Larmor frequency. This frequency lies in the microwave part of the electromagnetic spectrum. If, simultaneously, a constant magnetic field is imposed to induce precession, and an oscillating field of microwave frequency is applied, one should expect to observe a resonance effect (the atom absorbs energy resulting in its shifting from one energy level to another.) The frequency of the precession of the paramagnetic atom is equal to the Landé g factor (the ratio of the gyromagnetic ratio of the atom to that of an atom containing only orbital angular moments) times the Larmor frequency.

Substances possessing spontaneous magnetic moments, even in the absence of external magnetic fields, are called ferromagnetic. Such substances have a temperature of transition, called the Curie point, above which they are paramagnetic and ferromagnetism vanishes. The phenomenon of ferromagnetism is the resultant of the contribution from small regions within the specimen termed domains by Weiss. Within each domain, the local magnetization is saturated; however, the directions of magnetization of different domains need not necessarily be parallel. The process of magnetization is essentially causing the domains whose magnetic moment points in the direction of the applied field to grow, and those in other directions to shrink. A special case is the anti-ferromagnetism which is charaterized by an ordered anti-parallel arrangement of electron spins.

In the later part of the 19th century, a series of phenomena, now known as the thermomagnetic and galvanomagnetic effects were discovered in conductors. Recently, interest has been revived in their technological potentials, particularily since semiconductors and semimetals were found to exhibit these phenomena.

Both effects involve the interaction of magnetic fields with electric and thermal currents. The galvanomagnetic effect is a general nomenclature of two specific effects: 1. The Hall Effect, which is a transverse voltage difference when a magnetic field is applied perpendicularly to a longitudinal electric current; and 2. Ettinghausen Effect, which is a temperature difference appearing in a direction perpendicular to both longitudinal electric current and the applied magnetic field.

The thermomagnetic group encompasses two specific effects: 1. Nernst Effect is the transverse voltage difference that appears when a magnetic field is applied perpendicularly to a longitudinal thermal current, and 2. Righi-Leduc Effect is the transverse temperature difference observed where magnetic fields are applied perpendicularly to a longitudinal thermal current. Underlying these phenomena is the Lorentz force, i.e., the fundamental interaction between moving electric charges and magnetic fields.

An extension of Lorentz force accounts for the cyclotron phenomenon where current carriers in a solid are accelerated in spiral orbits about the axis of a static magnetic field. This phenomenon may prove of interest in substances with ring structure where the π-electron can be induced to assume orbital precession around the whole ring with a definite frequency which is a function of the field intensity.

Another application of Lorentz force has been exploited mainly in the process of extracting Na from NaCl for use as reactor coolant. Here the applied static field separates the charge carriers, i.e., ions, in the electrolyte.

Heat and Thermal Energy:

The interaction of thermal energy with a given system, on the molecular level is exhibited in forms of vibration, rotation, and excitation of electrons to higher energy levels. These responses are governed by quantum mechanical rules. In addition, and of particular importance from a biological viewpoint, thermal energy enhances rupture of chemical bonds.

An interesting characteristic of biological functions is the relatively narrow range of temperature over which these functions are allowed. Within this range there exists an optimal temperature at which a given function is performed with highest efficiency. Outside this optimal temperature, the efficiency of the system decreases and eventually, if the temperature is altered considerably, one encounters an irreversible loss of biological activities. Reviews by Johnson, Eyring and Polissar,(18) as well as Pollard,(19) discuss the relationship between biological functions and thermal energy.

In the section on magnetism we discussed breifly the relationship between magnetic energy and temperature. The thermal energy at room temperature is approximately 250 times that of magnetization by a field of 10 kG intensity. In other words, the thermal energy of a system at room temperature should overcome any alignment or orientation of paramagnetic centers. However, it should be born in mind that this argument is valid only for systems at equilibrium. In addition, this idealized argument uses for a model a system made of free atoms without accounting for interactions between these magnetic dipoles. Also, a degree of order, i.e., periodicity or quasi-periodicity in the structure of substances imposes limitations on the above argument.

Ionizing Radiation:

Perhaps the most striking effect of ionizing radiation upon biological systems is the disproportionality between the amount of energy deposited in the system through radiation and the observed end effect.

Ionizing radiation interacts with matter dissipating its energy by producing ionizations, excitations, dissociations, and free radicals. Heat is another product of such interaction but its amount is minute.

In an effort to explain the nature and dynamics of the effects of ionizing radiation upon biological systems, many theories were introduced, the earliest concerned themselves with the so called "direct action" of ionizing radiation. Later this approach had to be modified and the "indirect action hypothesis" emerged. A necessary assumption of this later hypothesis is the existence of diffusing agents induced by radiation in the surrounding medium; their effect is exerted by interacting with a sensitive site after diffusing into it. Since most biological systems contain large amounts of water, analysis of the possible importance of the decomposition products of water produced by radiation, such as the highly reactive radicals like HO_2, H and OH and molecules like H_2O_2, seemed appropriate especially since they exhibited oxygen dependence, a phenomenon of great importance in radiobiology. However, this argument necessitates a universal increase of the observed end effect of radiation with increasing the water content of the irradiated object, but in several biological systems, particularly plant seeds, experimental observations showed a reversed dependence.(20) These findings led to attributing only a part of the damage to products of water irradiation. More recently, a bulk of information showed that ionizing radiation induces the formation of free radicals in biochemically important organic compounds such as enzymes, amino acids as well as nucleic acids. Also, radicals were found in irradiated biological tissues.(20,21, 22,23,24,25,26,27,28,29,30,31) Ehrenberg, Ehrenberg and Zimmer(20) demonstrated the dependence of the number of paramagnetic centers on X-ray dose. There is a linear dependency for low doses which does not hold for high doses.(32) The nature and life time of these radicals depend on the irradiated system, the environmental parameters (oxygen, moisture, heat) during and after the irradiation process. It is worthwhile to note that the opinion held by many, that the absorption of radiation in biological material generally leads within microseconds to states stable in the physical sense, must be abandoned since it has been shown that free radicals may persist for weeks. Although it is not conclusive yet, evidence is mounting to incorporate free radicals in the process of radiation damage.

C. Chemical and Biological Factors.

Michaelis, and his associates, and Pauling were the first to introduce the concept of intermediate metastable products in some chemical reactions. Later these were found to possess paramagnetic nature. Commoner(8) showed that free radicals exist in living systems as they do in organic systems *in vitro*. He concluded that the existence of molecules or molecular complexes in the free radical state can be considered as a specific tissue characteristic. Metabolically active tissues exhibit the most intense spin resonance signals. Live bacteria possess spin centers. Microwave spectroscopy of this system showed that these centers are due to an organic free radical. Isenberg and Baird(6) have shown that such signals depend on the living state of the system, for when the bacteria were killed, these centers disappeared.

Muller, Hotz and Zimmer(10) reported the existence of spontaneous paramagnetism in bacteriophage. Blumenfeld(11) has shown that the magnetic properties of yeast undergo changes in the course of the culture growth and cell division. Amer and Camp(9) found that in developing Tribolium pupae, the nature as well as the intensity of spin resonance signal changed as a function of the time of the developmental path. Hollocher and Commoner(4) reported that free radicals are associated with mitochondrial particles; these are centers for enzymes and energy supply in the cell. Photosynthetic systems have been found to have paramagnetic centers when they undergo photosynthesis.(8)

It has been demonstrated by Imai, Hirai and Hashi(33) that the complex of enzyme-substrate had a paramagnetic molecular configuration. With the use of microwave spectroscopy, Hollocher and Commoner(4) have followed the transformation of succinate into fumarate by succinic dehydrogenase. They found that the concentration of the free radical was proportional to the activity of succinic dehydrogenase. The lack of hyperfine structure suggests that the unpaired electrons exist in a relatively large molecular system.

Since the oxygen molecule is a bi-radical with two unpaired electrons, it is expected to react easily and quickly with other radicals. When oxygen is present in a system and because of its paramagnetic nature, it may enable transitions from singlet to triplet molecular states which are normally forbidden. Such triplet states have comparatively long lives, and therefore the probability is increased that the molecules so excited may react.

In the discussion above, it was shown that free radicals exist in biological systems; moreover, they are also induced by ionizing radiation. The naturally occurring free radicals are generally encountered in active biological systems. Therefore, in selecting a system to test the role of magnetic fields in influencing biological phenomena, a reasonable choice is that of a dynamic system undergoing biochemical changes and which is sensitive to oxygen. Perhaps one of the more dynamic biological systems is one which is undergoing differentiation and development.

Hexapoda provide an interesting experimental tool for studying these two basic problems of biology in general and molecular biology in particular. For the transformation from the immature form to the adult capable of reproduction and which is of a totally different form and structure is quite striking. Furthermore, the process of metamorphosis, governed and controlled through endocrine activity which culminates with a drastic morphological change, differs from the developmental processes of other animals only in magnitude. Such a fact places the metamorphosing insect in a unique and attractive position from an experimental view point.

In the work described below, we chose the pupal state of the developmental path. The pupa is a very complicated and relatively closed system where mutually counteractive processes are maintained in harmonious sequence based upon a non-static hormonal balance. On one hand the juvenile hormone favors the synthesis of larval structures and opposes the achievement of adulthood; i.e., this hormone is a status-quo maintainer. On the other hand, the prothoracic gland hormone (ecdysone) is presumed to act directly upon the nuclear material resulting in the synthesis, through the proper ribonucleic acid (RNA), of enzymes which initiate the formation of adult tissue. Gilbert(34) proposed that, in an environment containing a <u>critical titer</u> of ecdysone and <u>specific</u> <u>concentration</u> of juvenile hormone, nuclear information is transferred to the cytoplasm leading to the histogenesis and differentiation of adult tissues; such a proposal is applicable to larval and pupal formation as well. The absence of the juvenile hormone is necessary for the formation of the adult.

In early pupal life there is a marked decrease of metabolism followed by a steep increase coupled with intense formation of mitochondria. It is well established that the rate of respiration during the pupal stage follows a U-shaped curve.(35,36) Agrell(37,38,39) found the spontaneous dehydrogenase activity as well as some other oxidative enzymes varies in the same U-shaped manner as the respiratory and metabolism curves.

Taking the metabolism of nucleic acids as an indication of protein synthesis, Agrell(40) in collaboration with Snygg found that the amount of deoxyribonucleic acid (DNA) did not change with respect to time during the pupal stage; however, the RNA in the water phase, which corresponds to the ribosomal RNA(41) did increase in amount during the differentiation of the pupa into adult.

The results reported below show that there is an interaction between applied magnetic fields and the kinetics of development and differentiation leading to alteration of developmental path. The degree and type of alteration are functions of the combination of physical and chemical parameters applied to the system.

II. MATERIALS AND METHODS

A. Materials:

1. The Biological System:

The biological system used in the experiments described in this paper is the pupa of Tribolium confusum. Much information about the biology of that system is available.(42,43,44,45,46,47) In culturing and handling Tribolium we followed the methods developed by Beck.(48,49)

2. X-irradiation:

250-kVp x-rays were used (Phillips 250-kV unit) and the beam was filtered with 1 mm. of aluminum. A uniform field was obtained at a distance of 5 cm. The dose rate at 15 milliamperes was approximately 1000 R/min. as measured by a Victoreen R-meter. The doses to be administered were monitored by a Radocon integrating dosimeter. Pupae were irradiated, in air at room temperature, in plastic petri dishes. Backscattering was negligible.

3. Magnetic Fields:

The applied magnetic fields were produced by permanent alnico V Horn type magnets of varying intensities. Each field was thoroughly scanned and mapped, using the method developed by Watson and Di Gregorio.(50) Inhomogeneity within the homogeneous part of each field did not exceed 0.01%. The control of each of the experiments described here was housed between the poles of a dummy magnet having the same geometry as the corresponding actual magnet.

4. Temperature Controlled Environment:

Strict control of the temperature of incubation is of absolute necessity. Fluctuations of temperature were limited, in time and space, to ±0.2°C. To achieve such close control over the fairly large volume which houses the experimental set up and its control, and to maintain it over the period of incubation (usually seven days); we had to modify our incubators (Precision Scientific Model 805) in two ways:

1. The temperature controller provided with the incubator was replaced by a YSI tele-thermometer temperature controller model 73. This unit has the dual benefit of providing a sensitive method for directly reading the temperature without disturbing the system, as well as controlling the temperature with adequate accuracy. The temperature is sensed by means of a thermistor probe which forms one leg of a d.c. Wheatstone bridge. The degree of the bridge imbalance is detected by a d.c. meter which is calibrated to indicate the temperature of the probe.
2. The inside of the incubators was lined with a double layer of PF105 fiber glass cemented to the walls and the floor minimizing the formation of air gaps. In addition, all incubators were kept in an air conditioned room, thus keeping to a minimum daily temperature fluctuation.

5. Temperature Recording and Monitoring:

Temperature of the environment of the experimental system and that of the control was monitored and recorded via two thermistors: one placed in the environment of the experimental container and the other in that of the control. Both thermistors were previously calibrated against an NBS calibrated mercury glass thermometer with 0.01°C sensitivity. The monitoring of the temperature was made by reading the temperature on the YSI tele-thermometer temperature controller model 73 mentioned in Section II, 4, which provided instantaneous reading of the temperature of both the control and the experimental system.

Since the YSI model 73 provides a direct means of temperature recording, the output of each thermistor, the experimental and the control, was fed into a YSI dual channel recorder type 81 for simultaneous and continuous temperature recording throughout the incubation period. The recorder is a galvanometer with a chopper bar pointer. An electronic flip flop presents each channel alternately to the recording mechanism. The chopper type DC amplifier is stabilized with negative feedback. The recorder provides calibrated full scale deflections for voltage as low as 10 millivolts, a range we chose to use.

6. Relative Humidity:

Since relative humidity did not constitute one of the variables in the experiments described in this paper, we merely monitored its value without attempting to control it. The relative humidity inside the temperature controlled chambers was between 35% and 40%.

B. Experimental Methods:

In all experiments described below, pupae eight or less hours of age were used; by this we mean that pupae were collected for experimental use eight hours after the cultures had been cleared of all pupae. Although the radio- and thermosensitive period of the pupa is the first 32 hours of the pupal life, in an attempt to achieve partial synchrony of the biochemical events in the pupae and without sacrificing the pupal yield of the cultures, we chose to harvest at eight hour intervals.

Exposure to magnetic fields was continuous throughout the pupal stage, and scoring for data was executed at the end of that stage.

1. Experiments to Test the Effect of Magnetic Fields upon the Development of Radiation Injury:

In this set of experiments pupae received at room temperature a dose of 1200 R of x-rays. Immediately, the irradiated population was divided into two halves: one constituted the experimental group and was placed in glass container whose dimensions were that of the homogeneous part of the magnetic field. The container was then placed in a magnetic field of a given intensity; the other half of the irradiated pupae served as the control and similarly was placed in a glass container having the same geometry as that of the experimental container. The control container was then placed between the poles of the dummy magnet. A thermistor was placed in each container for continuous temperature monitoring. Both the actual and the dummy magnets were housed in the same incubator which was sealed for the length of the pupal period, at the end of which the experimental system, now adult, is scored for the criterion of wing anomaly (which is the end point of that particular set of experiments.) A description of this criterion is given in Section II,C. The incubation temperature was $38.0^{\circ}C \pm 0.2$. These experiments were performed with varied field intensities: 2.2, 3.6, 6.6, 8.0, 9.7 kG. (Fig. 1).

2. Experiments to Investigate the Conbined Effects of Higher Incubation Temperature and Magnetic Field upon the Development of Tribolium pupa:

The purpose of this group of experiments is to test the effects of magnetic fields when combined with critically high temperature of incubation, $39.5^{\circ}C \pm 0.2$, upon the kinetics of developmental processes in the pupa. Two groups of pupae were incubated at this higher temperature, one in the homogeneous part of a field of a given intensity and the other serving as its control. Temperature was monitored continuously in both groups and the exposure to the fields was continuous throughout the pupal stage. The experiments were performed with the magnetic field intensities mentioned in Section II, B, 1. The end point for this set of experiments was the criterion

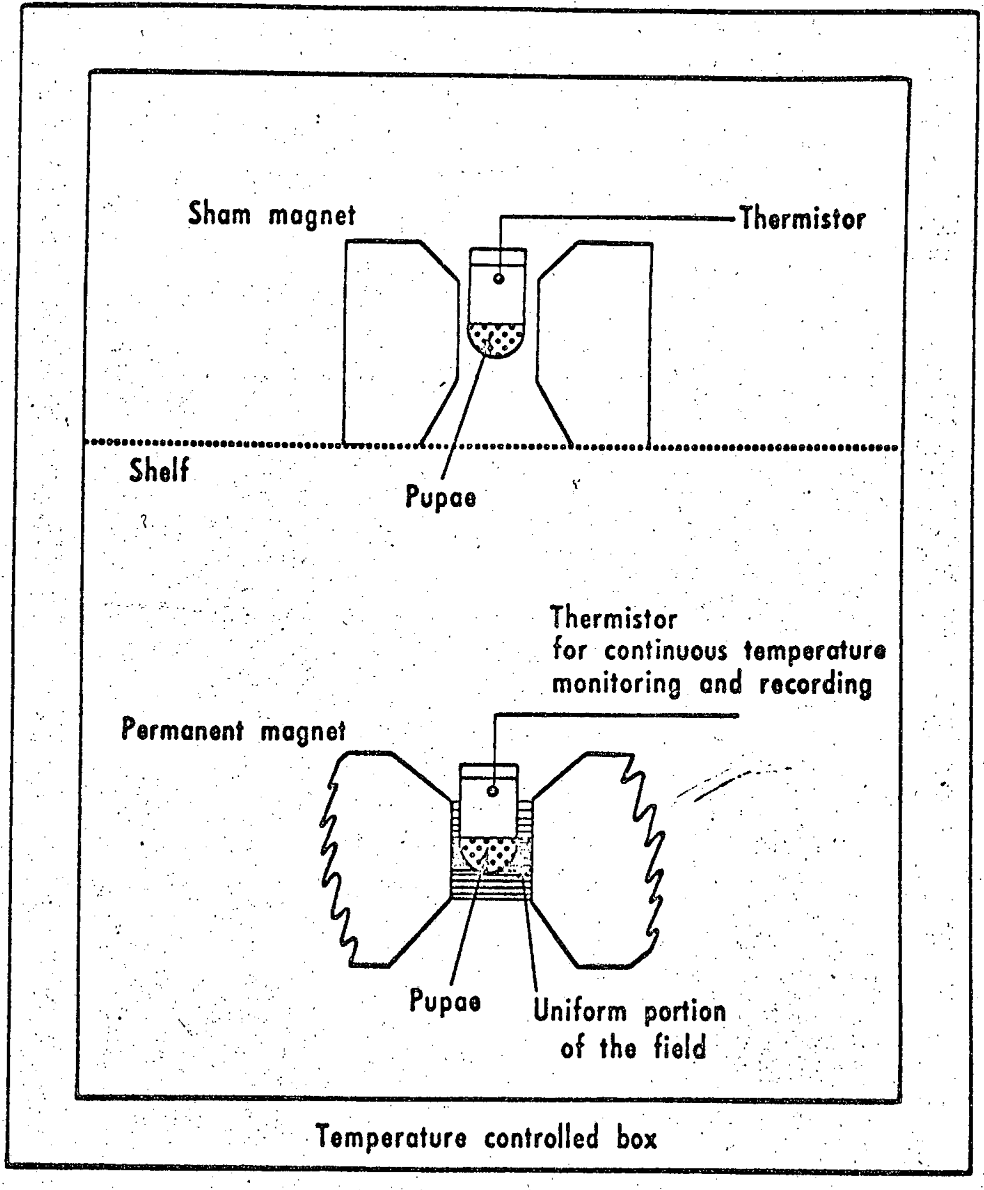

Figure 1.

MU.37031

of molting failure, a description of which is given in Section II,C.

3. Experiments to Study the Combined Effects of Temperature, Oxygen Tension and Magnetic Fields upon the Development of Tribolium Pupae:

Pupae were incubated in magnetic fields of varied intensities, at different temperatures: 38.5°C ± 0.2, and 39.0°C ± 0.2. Different oxygen tensions in the ambient environment of the system constituted the additional variable. The system was flushed with the proper gas mixture once every 12 hours throughout the pupal period. Thus we assured the presence of an ample amount of the specific ambient atmosphere with minimal disturbance of the temperature of incubation. The flushing time was a function of the respiration rate at different temperatures, the number of animals, the volume of the container as well as the rate of flushing. Temperature was monitored continuously. The criterion of this set of experiments was the incidence of molting failure. The field intensity was 6.3 kG. The controls were kept in ambient environment made of the following oxygen percentages in nitrogen: 2 1/2%, 5%, 7 1/2%, 10%, 15%, 21%, 30%, 40%, 60%, 80%, and pure oxygen. The results from the control experiments led us to limit the experimental groups to ambient compositions of 15%, 21%, 30%, 40%, and 60% oxygen in nitrogen. The percentages mentioned above have a range of ± 0.5% (Fig. 2).

4. Experiments to Investigate the Combined Effects of X-irradiation, Oxygen Tension and Magnetic Fields upon the Development of Tribolium Pupae:

Radiosensitive pupae received a dose of 900 R of 250 kVp x-ray photons. Immediately following irradiation, the system was treated in similar manner as in Section II,B,3. The ambient tension of oxygen was: 15%, 21%, 30% and 40% in nitrogen. The incubation temperature was 38.5°C ± 0.2. The experimental criterion was the wing anomaly.

C. Experimental Criteria:

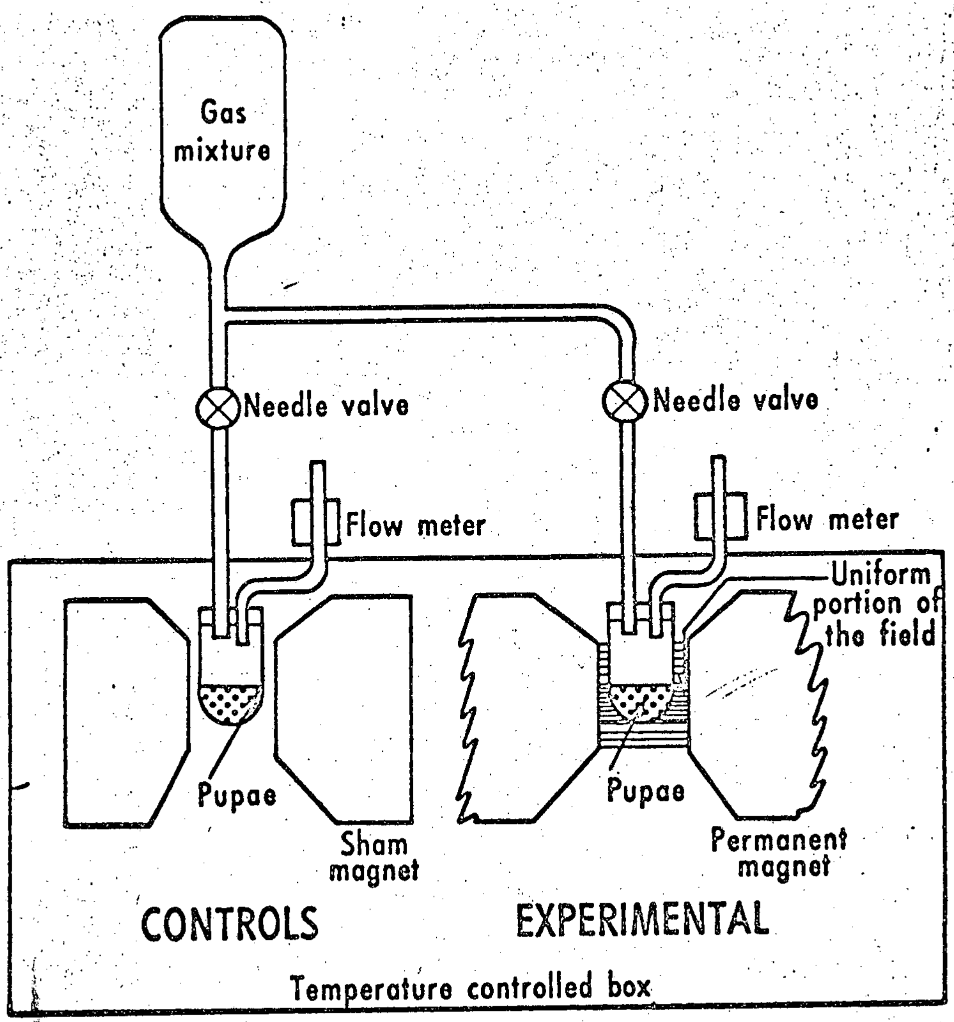

Figure 2.

MU-37030

C. Experimental Criteria:

As mentioned earlier, the physical and chemical parameters were applied to the biological system during its pupal period. The elicited effects of of these parameters were sought at the end of the incubation time (7 days) through three criteria:

1. Wing Anomaly:

The anomaly of the adult wings is defined(48,49) as: 1.) Deformity of the external wings (the elytra) which appears as a slight irregular warping, and a displacement dorsally and laterally from the normal position over the membranous wings and the dorsolateral aspect of the abdomen. 2.) Deformity of the membranous wings which is externally manifested by warping, failure to fold after eclosion, and lateral displacement from normal position over the dorsal aspect of the abdomen. Though the deformity appears to be specific; it is not an all-or-none phenomenon. Figure 3 shows pictures of normal wings and typical deformed ones.

2. Molting Failure:

Assuming the process of development of the adult from the pupa is a continuous one, molting failures are then classified as pupae that have progressed on the developmental scale but failed to change into adults. By progress on the developmental scale we mean the ability to form the following adult characteristics, which usually takes place during the last 24 hours of the pupal age: 1.) Formation of the complex eyes denoted by brown coloration, 2.) Distinct formation and pigmentation of the mouth parts, and, 3.) Pigmentation of the limbs. Molting failures die with these characteristics and without undergoing the final and last step of development, i.e., formation of the adult. (Fig. 4)

3. Pupal Death:

At the end of the incubation time, pupae which do not show any external

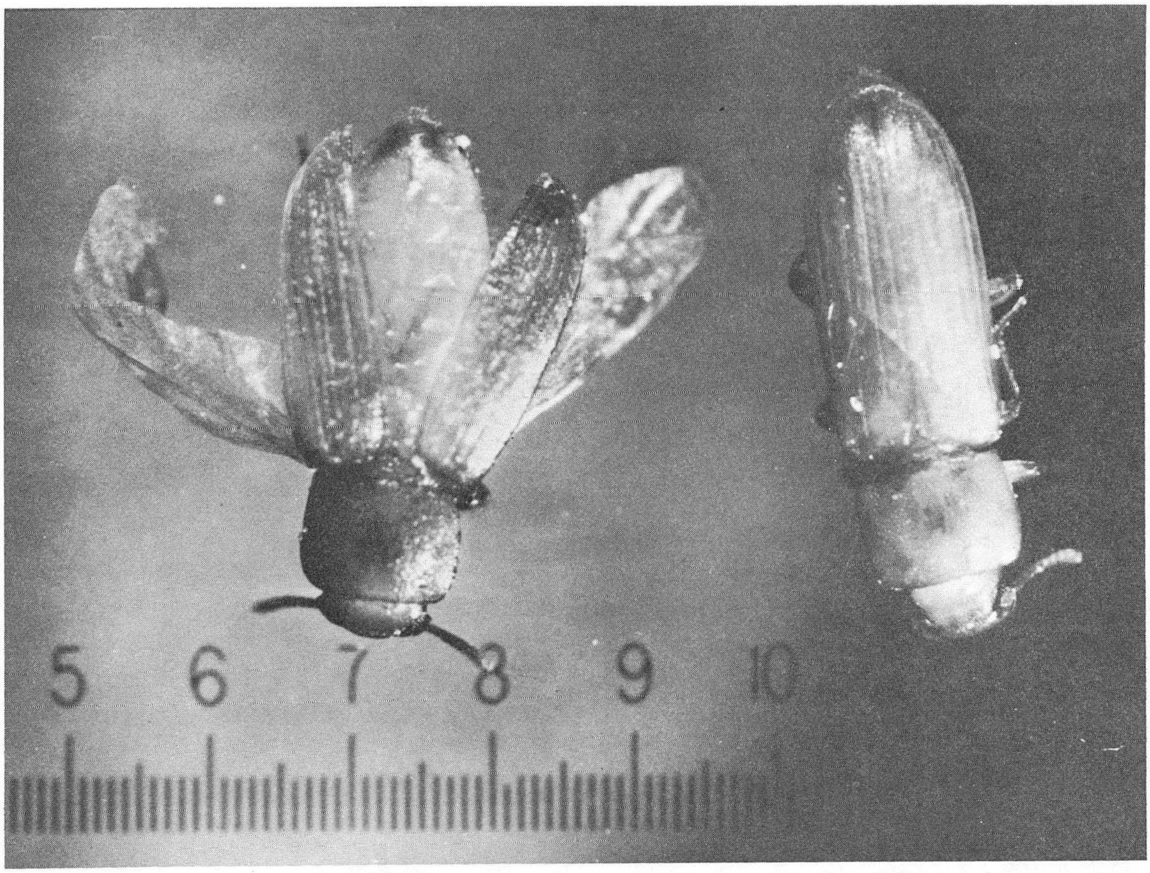

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Figure 3. (Scale: smallest division = 0.1 mm.)

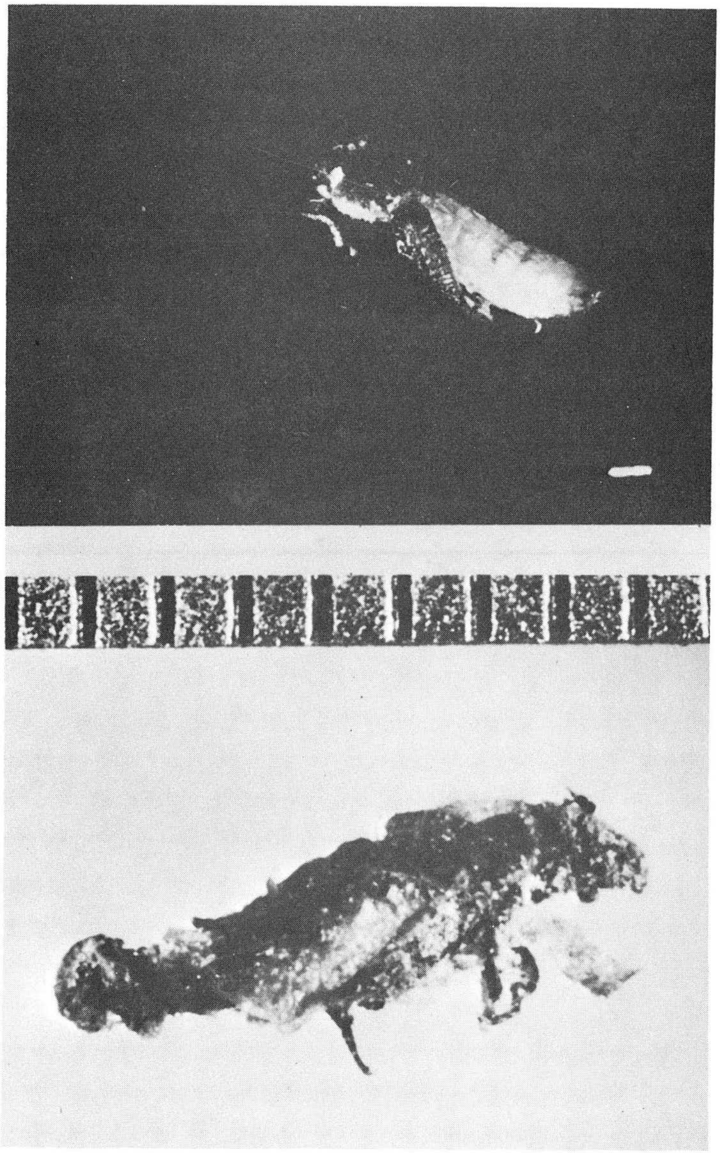

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Figure 4. (Scale in lower photograph: smallest division = 0.5 mm.)

morphological differences from those of 8 hours of age, were classified as pupal death. It appears they were arrested, early in the pupal period, from undergoing development. (Fig. 5)

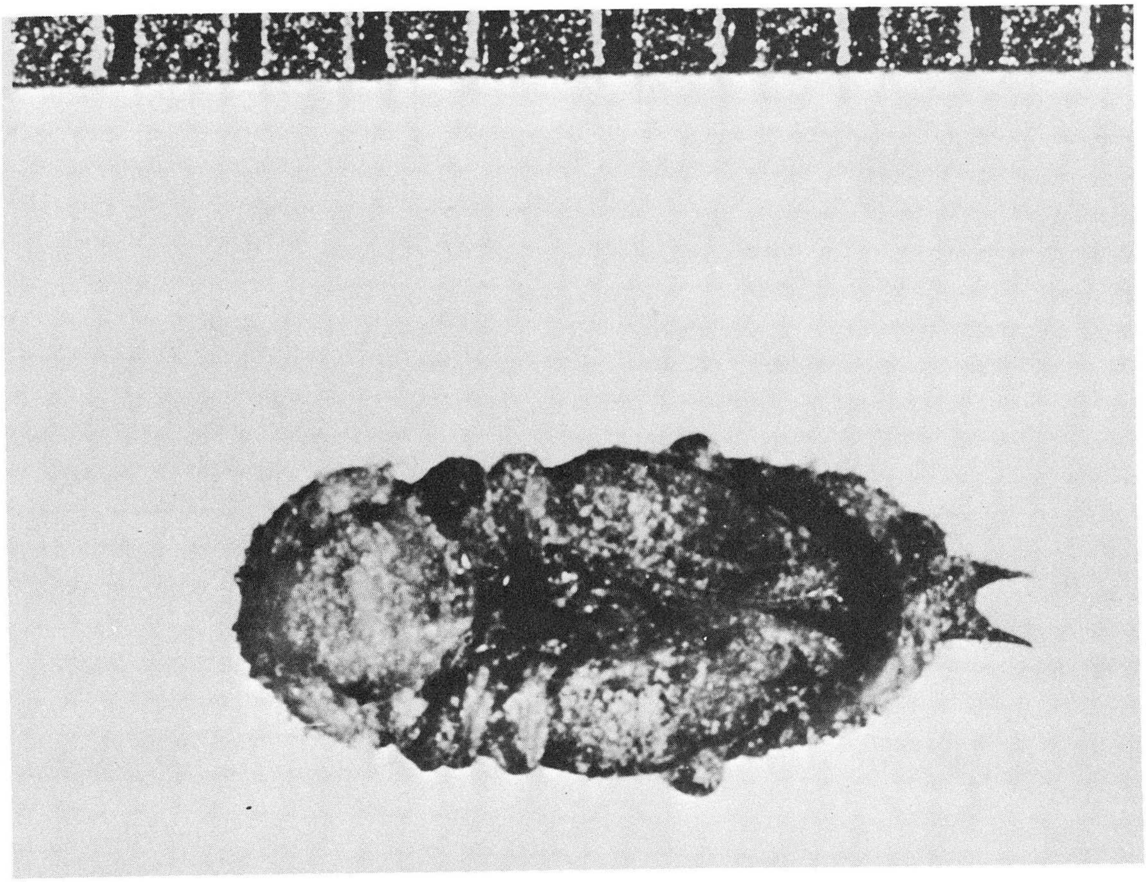

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Figure 5. (Scale: smallest division = 0.5 mm.)

III. RESULTS

A. Temperature Dependence of the Development of Tribolium Pupae:

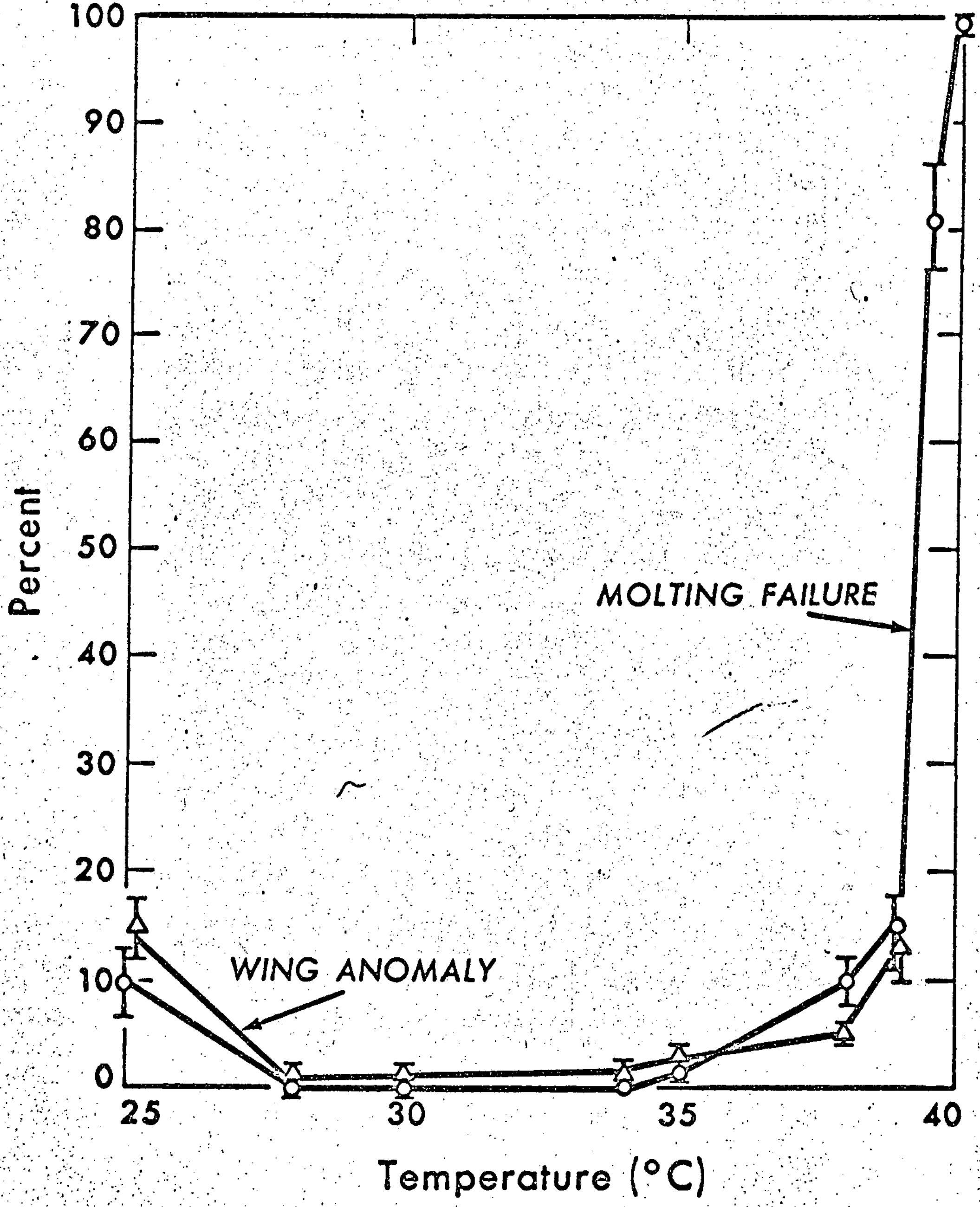
Tribolium pupae live in an optimum temperature range of 28° to 33°C. At that range most pupae complete the molting process in about a week and become normal adults. Lower and higher temperatures are hazardous but for different reasons: at lower temperatures, the rate of eclosion is markedly slowed (at 20°C to 3 weeks), yielding a high fraction of insects that developed abnormally. At higher temperatures, abnormal development occurs with increasing frequency. At 40°C all animals fail to eclose and die as molting failures. (Fig. 6 and Tables I and II). Between 39°C and 40°C there is a steep rise in the fraction of molting failures.

B. Synergism Between Temperature and Radiation:

Amer *et. al.*(51,52) showed the existence of synergistic action between elevated temperature and ionizing radiation upon the process of the induction of wing anomalies in Tribolium. A dose of 1200 R of X-rays followed by incubation at the optimal temperature of 30°C results in 15% wing anomalies. Incubation at 38°C, and without radiation, results in 5% wing anomalies. When radiation and incubation at 38°C are combined, the percentage of wing anomaly is about 70%. This three-and-a-half fold increase provides a sensitive biological amplifier enabling the detection of small responses.

C. Initial Observations of the Combined Effects of Radiation, Temperature and Magnetic Fields:

In February 1963 a set of pilot experiments were executed by the author to test the effect of magnetic fields upon the development of irradiated Tribolium pupae when incubated at 38°C and 30°C. The magnetic field intensity used was 3.6 kG, and the radiation doses were 1200 R for incubation at 38°C and 1500 R for incubation at 30°C (both doses result in the same percentage



MU-37024

Figure 6.

TABLE I: Temperature Dependence of Molting Failure

Experiment	Number of Animals per Column	Percent Molting Failure* at 25°C	28°C	30°C	34°C	35°C	39°C	39.5°C	40°C
1	300	9.7±1.7**	0.0±0.0	0.0±0.0	0.7±0.5	1.0±0.6	33.0±2.7	76.7±2.4	100.0±0.0
2	300	11.1±1.8	0.0±0.0	0.0±0.0	0.0±0.0	0.3±0.3	29.0±2.6	84.3±2.1	100.0±0.0
3	300	10.0±1.7	0.0±0.0	0.3±0.3	0.0±0.0	1.0±0.6	35.3±2.7	79.3±2.3	99.0±0.6
4	300	6.3±1.4	0.3±0.3	0.3±0.3	0.0±0.0	0.7±0.5	27.7±2.6	86.7±2.0	98.7±0.6
5	300	12.7±1.9	0.7±0.5	0.0±0.0	0.3±0.3	1.7±0.7	29.3±2.6	72.7±2.6	100.0±0.0
6	300	13.0±1.9	0.0±0.0	0.0±0.0	0.0±0.0	2.0±0.8	37.3±2.8	81.6±2.2	99.3±0.5
7	300	8.3±1.6	0.0±0.0	0.0±0.0	0.0±0.0	3.0±1.0	32.6±2.7	77.3±2.4	98.7±0.6
8	300	9.8±1.7	0.0±0.0	0.0±0.0	0.7±0.5	2.7±0.9	30.7±2.6	89.6±1.8	100.0±0.0
9	300	11.7±1.8	0.0±0.0	0.0±0.0	0.0±0.0	2.0±0.8	23.3±2.4	92.3±1.5	99.3±0.5
10	300	7.4±1.5	0.0±0.0	0.3±0.3	0.3±0.3	0.7±0.5	26.7±2.5	74.3±2.5	100.0±0.0
Mean of all experiments ± s.d. of the mean***		10.0±1.7	0.1±0.18	0.1±0.16	0.2±0.26	1.5±0.7	30.0±2.6	81.0±2.2	99.5±0.4

* Percent Molting Failure = $\frac{\text{Total Number of Molting Failures}}{\text{Total Number of Animals}} \times 100$.

** s.d. was computed from the formula: $\sigma = \sqrt{\frac{f(1-f)}{n}}$ where f is the fraction of deformed animals and n is the total number of animals.

*** s.d. of the mean was computed from the formula $\sigma_{\bar{x}} = \sqrt{\frac{\Sigma \sigma^2}{n-1}}$, where n is the number of experiments.

TABLE II: Temperature Dependence of Wing Anomaly

Experiment	Number of Animals per Column	Percent Wing Anomaly* at 25°C	28°C	30°C	34°C	35°C	38°C	39°C
1	300	20.7±2.3	0.7±0.5	2.7±0.9	1.7±0.7	3.3±1.0	5.0±1.3	10.0±1.7
2	300	13.0±1.9	3.0±1.0	0.7±0.5	1.0±0.6	3.6±1.1	5.3±1.3	17.7±2.2
3	300	15.7±2.1	1.0±0.6	0.3±0.3	1.0±0.6	1.7±0.7	3.7±1.1	19.0±2.3
4	300	19.7±2.3	2.0±0.8	3.0±1.0	3.3±1.0	2.7±0.9	6.7±1.4	9.7±1.7
5	300	11.7±1.7	1.7±0.7	1.7±0.7	1.7±0.7	2.0±0.8	7.0±1.5	7.0±1.5
6	300	10.0±1.7	0.7±0.5	1.0±0.6	0.7±0.5	2.7±0.9	3.0±1.0	13.0±1.9
7	300	18.0±2.2	1.0±0.6	2.7±0.9	2.0±0.8	3.0±1.0	5.0±1.3	15.0±2.0
8	300	19.0±2.3	2.6±0.9	1.0±0.6	2.6±0.9	3.7±1.1	5.3±1.3	6.7±1.4
9	291	10.7+1.8	0.3±0.3	0.4±0.3	3.3±1.0	2.6±0.9	3.5±1.0	13.0±2.0
10	295	12.3±1.9	2.0±0.8	1.9±0.8	2.7±0.9	4.7±1.2	5.7±1.3	19.6±2.3
Mean of all experiments ± s.d. of the mean		15.0±2.0	1.5±0.7	1.5±0.7	2.0±0.8	3.0±1.0	5.0±1.3	13.0±1.9

$$* \text{ Percent Wing Anomaly} = \frac{\text{Number of Animals with Wing Anomaly}}{\text{Total Number of Adults}} \times 100 .$$

of wing anomaly.) Positive results were obtained from experiments where pupae were incubated at 38°C: the wing anomaly frequency was reduced from 70% to about 40%. Results from incubation at 30°C did not show an appreciable difference between the experimental groups and their corresponding controls. Further experiments showed a definite and crucial dependence of positive results upon temperature fluctuation during the incubation period and subsequently led to a more sophisticated system of temperature control and monitoring.

D. Combined Effects of Radiation, Incubation at 38°C and Exposure to Magnetic Fields:

Figure 7 and Table III show the incidence of wing anomaly as a function of the applied magnetic field. It is evident that the percentage of radiation-induced wing anomaly decreases steadily with the increase in the field intensity up to 8 kG; at 9.7 kG the incidence of anomalies increases again. To verify this observed rise, and to eliminate the possibility that such rise is attributed in some way to the small gap of the 9.7 kG magnet (an imposed limitation in permanent magnets when high fields are desired), we tested the effects of the same field intensity, produced by electromagnet where larger gaps are accessible. The results of these tests confirmed the observed increase. (Table IIIa)

These findings suggest that the magnetic field has a biphasic mode of action upon the frequency of damage caused by the synergistic action between radiation and elevated temperatures. At field intensities up to 8 kG the increase in field intensity is systematically accompanied by an increase in the rate of "protection" against the type of damage described above. At 9.7 kG the experimental evidence leads to the conclusion that in the higher region of field intensities the "protective rate" of magnetic

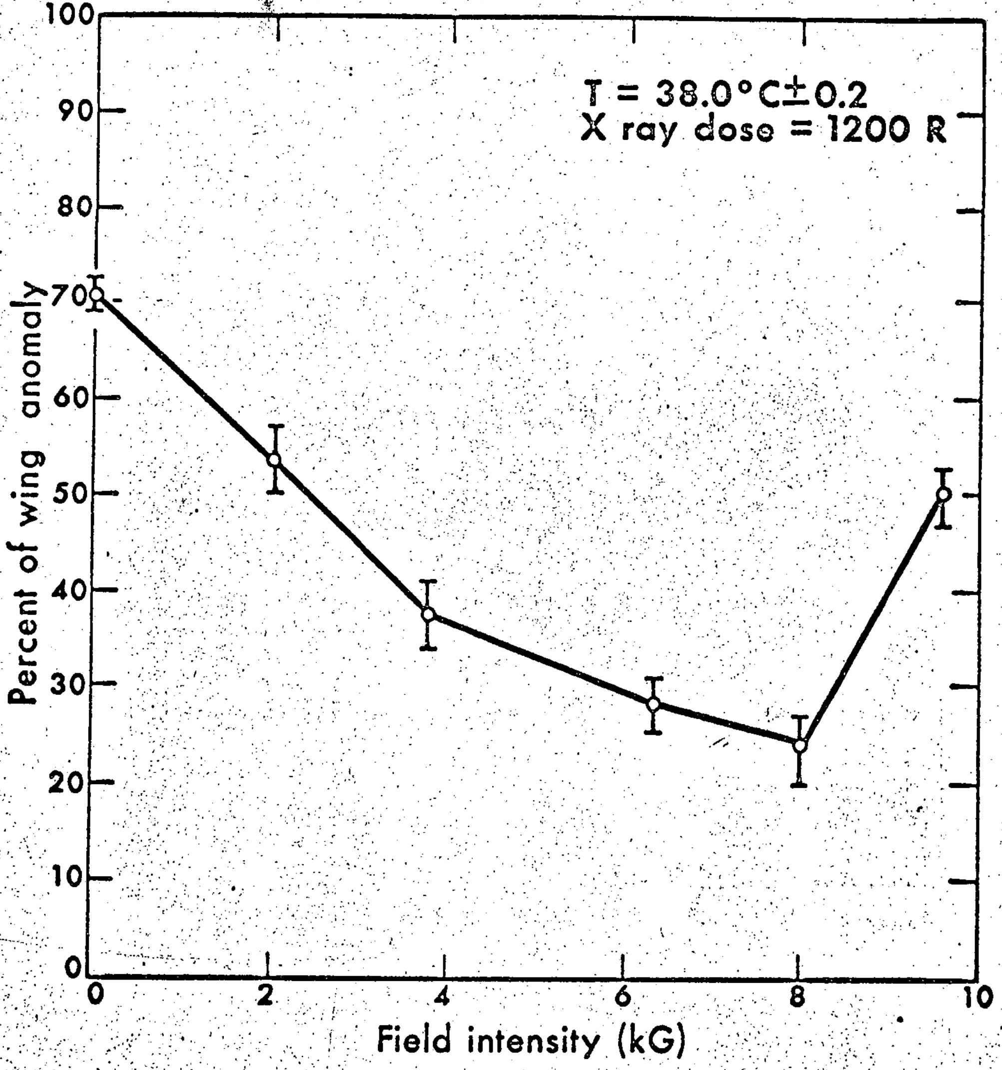


Figure 7.

MU-37025

TABLE III: Magnetic Field Intensity Dependence of Wing Anomaly*

Experiment	Number of Animals per Column	Percent Wing Anomaly at Field Intensity of					
		2.2 kG	3.6 kG	6.6 kG	8.0 kG	9.7 kG	Control
1	298	47.7±3.0	39.0±2.8	33.0±2.7	32.3±2.7	42.7±2.9	67.0±2.7
2	300	57.7±2.8	43.3±2.8	30.3±2.6	25.0±2.5	49.0±2.9	73.3±2.6
3	300	59.0±2.8	39.7±2.8	27.7±2.6	23.3±2.4	53.3±2.9	78.0±2.4
4	293	46.3±2.9	35.7±2.8	21.7±2.4	16.0±2.1	58.0±2.8	68.7±2.7
5	300	55.0±2.9	32.0±2.7	25.0±2.5	25.0±2.5	50.7±2.9	72.0±2.6
6	300	56.3±2.9	46.0±2.9	29.7±2.6	25.7±2.5	56.0±2.9	71.7±2.6
7	300	51.7±2.9	36.0±2.8	30.0±2.6	26.7±2.6	53.3±2.9	69.0±2.7
8	295	57.0±2.9	39.7±2.8	23.3±2.4	19.7±2.3	50.3±2.9	65.7±2.7
9	300	46.7±2.9	34.0±2.7	33.0±2.7	17.7±2.2	46.7±2.9	70.0±2.6
10	299	58.0±2.8	38.3±2.8	31.7±2.7	28.7±2.6	43.0±2.9	75.0±2.5
Mean of all experiments ± s.d. of the mean		53.5±2.9	38.0±2.8	28.5±2.6	24.0±2.4	50.3±2.9	71.0±2.6

* Radiation dose: 1200 R ; Incubation temperature: 38.0°C ± 0.2 .

TABLE IIIa

Verification of the Relative Rise in Wing Anomaly Observed at 9.7 kG*

Experiment	Number of Animals	Percent Wing Anomaly at Field Intensity of 9.7 kG (Produced by Electromagnet)
1	400	55.8 ± 2.5
2	400	48.3 ± 2.5
3	400	49.1 ± 2.5
Mean of all experiments ± s.d. of the mean		51.1 ± 3.1

* Radiation dose: 1200 R

Incubation Temperature: 38.0°C ± 0.2 .

fields is reduced. The need for exploring the response at higher fields ($>$10 kG)is obvious.

E. Combined Effects of Higher Incubation Temperature and Magnetic Fields:

Since the decrease of incidence of wing anomaly described above is similar to what is obtained following incubation at slightly lower temperatures, the experiments described in Section II,B,2, were performed to test the hypothesis that the effects of the external magnetic fields are equivalent, qualitatively, with that obtained with cooling.

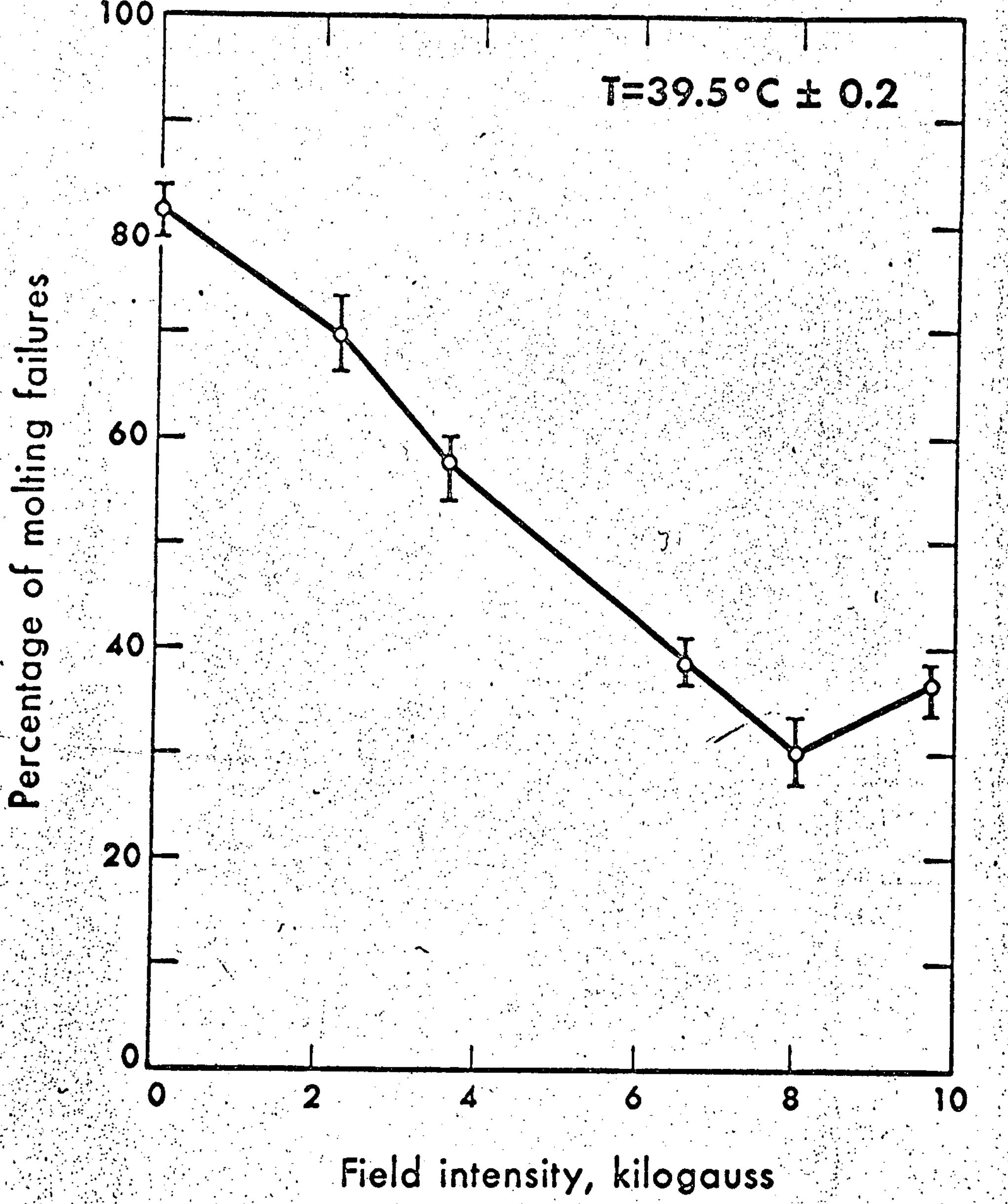
Figure 8 and Table IV describe the relationship between molting failures and the intensity of the applied field. In the absence of the field about 80% of the pupae fail to develop and die as molting failures. The application of magnetic fields resulted in a striking decrease of molting failure incidence coupled with increase of the survivals. The degree of reduction in molting failures is maximum at 8 kG and levles off at higher fields.

It is evident from these results that magnetic fields afford some protection against heat-induced damage.

F. Combined Effects of Temperature, Oxygen Tension and Magnetic Fields upon Development of Tribolium Pupae:

At one atmospheric pressure, the optimum ambient oxygen concentration at 30°C. is between 21-30% with a normal range of 15-40%, and normal development is usually conplete. At lower and higher partial pressures molting and eclosion are incomplete and anomalies are more frequent. Low oxygen tension leads to anoxic death, whereas at high oxygen tension oxygen toxicity sets in.

As shown in Figures 9 and 10, and Tables V and VI, the tolerable range of oxygen concentration for molting failures decreases as temperature increases, and the U-shaped curves tend to assume V-shapes accompanied by an upward



MU-37029

Figure 8.

TABLE IV: Magnetic Field Intensity Dependence of Molting Failure*

Experiment	Number of Animals per Column	Percent Molting Failure at Field Intensity of 2.2 kG	3.6 kG	6.6 kG	8.0 kG	9.7 kG	Control
1	300	77.0±2.4	62.0±2.8	35.7±2.7	29.3±2.6	35.3±2.7	72.6±2.6
2	298	68.3±2.7	55.0±2.9	33.0±2.7	35.0±2.7	38.3±2.8	89.3±1.8
3	300	69.7±2.7	53.3±2.9	32.0±2.7	32.7±2.7	32.7±2.7	76.0±2.4
4	300	63.0±2.8	60.0±2.8	37.7±2.8	26.7±2.5	32.0±2.7	77.7±2.4
5	290	71.7±2.6	68.3±2.7	39.7±2.8	33.0±2.7	38.0±2.8	93.0±1.4
6	300	66.0±2.7	35.0±2.9	36.0±2.8	29.6±2.6	40.0±2.8	74.6±2.5
7	300	70.7±2.6	57.0±2.9	41.7±2.8	30.6±2.6	40.3±2.8	79.6±2.3
8	289	72.0±2.6	49.3±2.9	30.0±2.6	22.7±2.5	30.0±2.6	86.7±2.0
9	300	73.0±2.6	63.7±2.8	46.3±2.9	37.0±2.8	43.0±2.8	81.7±2.2
10	300	63.7±2.8	51.7±2.9	43.0±2.9	27.7±2.6	31.7±2.7	84.6±2.1
Mean of all experiments ± s.d. of the mean		69.5±2.6	57.5±2.8	37.5±2.8	30.4±2.7	36.1±2.6	81.6±2.3

* Temperature of Incubation: 39.5°C ± 0.2 .

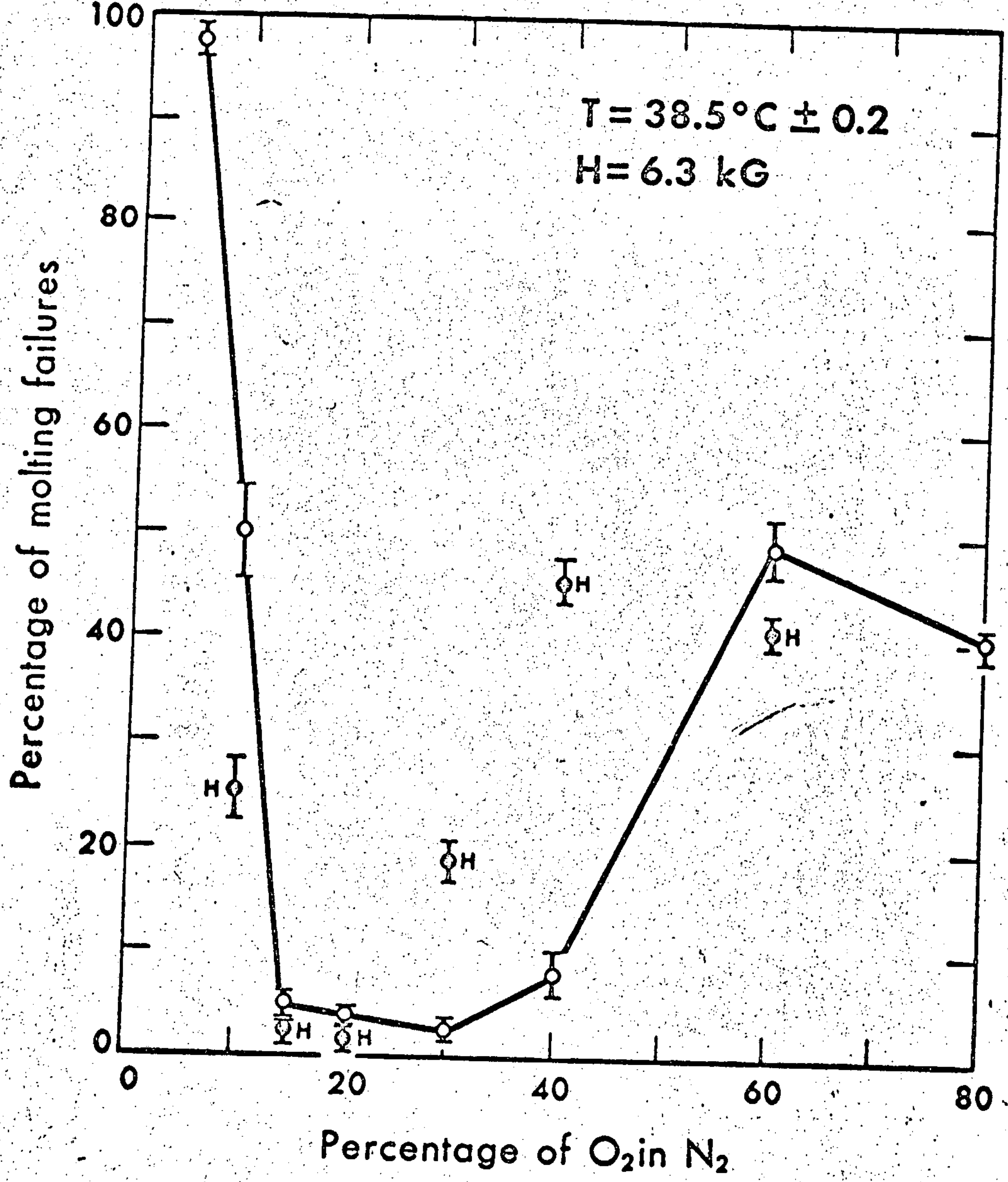


Figure 9.

MU-37028

(The decrease in incidence of molting failures at oxygen tensions greater than 60% is due to corresponding increase in the incidence of pupal deaths.)

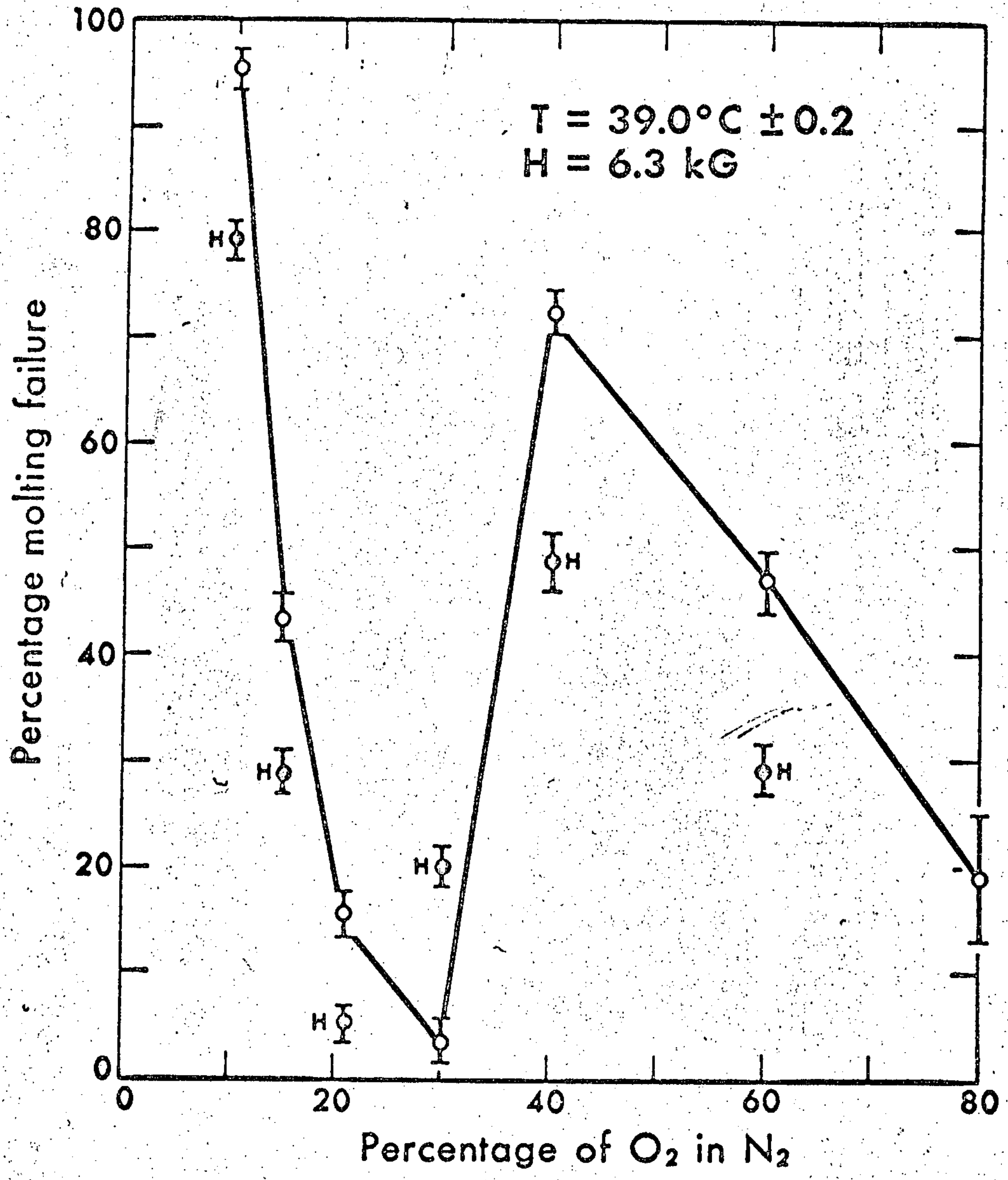


Figure 10. MU-37027

(The decrease in incidence of molting failures at oxygen tensions greater than 40% is due to corresponding increase in the incidence of pupal deaths.)

TABLE V: Combined Effects of Magnetic Fields*, Ambient Gas Composition and Incubation at 38.5°C ± 0.2 upon Molting Failure

Experiment	No. of Animals /Column	Percent Molting Failure at Gas Composition of											
		10% Oxygen		10% Oxygen		21% Oxygen		30% Oxygen		40% Oxygen		60% Oxygen	
		C**	H***	C	H	C	H	C	H	C	H	C	H
1	300	43.3±2.9	18.3±2.2	4.0±1.1	2.0±0.8	3.0±1.0	0.3±0.3	1.3±0.6	18.0±2.2	10.0±1.7	51.0±2.9	57.0±2.9	46.0±2.9
2	300	53.0±2.9	30.7±2.6	6.3±1.4	3.3±1.0	2.3±0.8	0.7±0.5	1.3±0.6	19.7±2.3	5.3±1.3	45.7±2.9	47.3±2.9	40.3±2.8
3	300	56.7±2.9	36.7±2.8	6.7±1.5	3.0±1.0	3.3±1.0	1.7±0.7	2.0±0.8	25.0±2.5	5.7±1.3	39.7±2.8	39.7±2.8	32.0±2.7
4	300	40.7±2.8	18.0±2.2	5.0±1.3	1.3±0.6	4.0±1.1	2.7±0.9	2.0±0.8	19.3±2.3	9.0±1.6	41.0±2.8	45.0±2.9	36.0±2.8
5	300	59.0±2.8	32.7±2.7	2.7±1.0	2.3±0.9	3.7±1.1	0.7±0.5	1.7±0.7	14.3±2.0	8.7±1.6	41.0±2.8	51.0±2.9	39.7±2.3
6	300	50.7±2.9	22.3±2.3	5.3±1.3	2.3±0.9	2.0±0.8	1.0±0.6	2.7±0.9	16.7±2.2	4.7±1.3	39.7±2.8	49.3±2.9	42.0±2.9
7	300	52.1±2.9	29.0±2.6	5.0±1.3	3.9±1.1	3.0±1.0	1.0±0.6	3.0±1.0	22.7±2.3	4.0±1.1	46.0±2.9	53.7±2.9	49.0±2.9
8	300	58.3±2.8	31.3±2.6	5.7±1.3	3.0±1.0	5.3±1.3	3.0±1.0	2.7±0.9	18.0±2.1	12.0±1.9	50.0±2.9	45.3±2.9	42.0±2.8
9	300	46.0±2.9	19.7±2.3	3.0±1.0	3.0±1.0	4.7±1.3	2.0±0.8	2.7±0.9	20.3±2.3	11.7±1.9	49.3±2.9	56.7±2.9	43.0±2.9
10	300	42.0±2.8	17.0±2.1	6.0±1.4	3.3±1.0	4.0±1.1	2.3±0.9	3.7±1.1	17.7±2.2	11.7±1.9	52.0±2.9	48.0±2.9	43.3±2.9
Mean of all experiments ± s.d. of the mean		50.1±2.9	25.5±2.4	5.0±1.3	2.7±0.9	3.5±1.1	1.5±0.7	2.3±0.8	19.1±2.2	8.3±1.6	45.5±2.9	49.3±2.9	41.4±2.8

* Field Intensity: 6.3 kG

** C: data from the control of a given oxygen tension

*** H: data from the magneto-treated group for a given oxygen tension

TABLE VI: Combined Effects of Magnetic Fields*, Ambient Gas Composition and Incubation at 39.0°C ± 0.2 upon Molting Failure

Experiment	No. of Animals /Column	Percent Molting Failure at Gas Composition of											
		10% Oxygen		15% Oxygen		21% Oxygen		30% Oxygen		40% Oxygen		60% Oxygen	
		C	H	C	H	C	H	C	H	C	H	C	H
1	300	100.0±0.0	76.6±2.4	43.3±2.9	31.3±2.6	10.7±1.8	3.7±1.0	2.7±0.9	20.7±2.3	75.3±2.5	48.0±2.9	50.0±2.9	35.0±2.6
2	300	97.0±1.0	72.0±2.6	32.7±2.7	19.7±2.3	21.0±2.3	6.3±1.4	3.7±1.1	26.0±2.5	73.0±2.6	50.3±2.9	44.0±2.9	29.3±2.6
3	300	95.0±1.2	92.6±1.5	40.0±2.8	28.7±2.6	15.7±2.1	5.0±1.3	3.7±1.1	16.3±2.2	67.7±2.7	41.7±2.9	49.7±2.9	36.7±2.8
4	300	89.7±1.7	72.3±2.6	49.3±2.9	37.0±2.8	13.0±1.9	3.3±1.1	3.0±1.0	18.0±2.2	68.7±2.7	46.3±2.9	52.3±2.9	31.0±2.6
5	300	100.0±0.0	83.7±2.1	36.0±2.8	20.0±2.3	18.7±2.3	5.7±1.3	2.7±0.9	13.7±2.0	66.3±2.7	47.0±2.9	43.0±2.9	33.7±2.3
6	300	100.0±0.0	81.3±2.2	35.0±2.8	17.0±1.9	12.0±1.9	5.0±1.3	4.0±1.1	23.0±2.6	70.0±2.6	44.7±2.9	37.0±2.8	24.0±2.4
7	300	99.0±0.5	74.6±2.5	43.3±2.9	30.0±2.6	17.7±2.2	5.3±1.3	4.0±1.1	19.3±2.3	72.0±2.6	51.7±2.9	42.3±2.8	23.0±2.4
8	300	91.3±1.6	77.6±2.4	41.0±2.8	32.0±2.7	15.0±2.1	5.7±1.3	3.3±1.0	21.0±2.3	72.7±2.6	54.3±2.9	51.3±2.9	28.0±2.6
9	300	93.7±1.4	78.6±2.4	49.7±2.9	35.7±2.7	19.0±2.3	6.7±1.4	3.7±1.1	18.7±2.2	74.0±2.6	53.7±2.9	46.0±2.8	26.7±2.5
10	300	91.3±1.6	70.3±2.7	49.7±2.9	33.0±2.7	13.3±1.9	3.0±1.0	4.3±1.2	23.3±2.6	80.3±2.3	57.0±2.8	50.0±2.9	32.3±2.7
Mean of all experiments ± s.d. of the mean		95.7±1.1	77.9±2.3	42.0±2.8	28.4±2.5	15.6±2.1	5.0±1.3	3.5±1.0	20.1±2.3	72.0±2.6	48.4±2.9	46.5±2.9	30.0±2.6

* Field Intensity: 6.3 kG

displacement. The application of magnetic fields results in a shift of the curve to the left. This shift leads to the inference that the external field enhances the effectiveness of oxygen. This response was more pronounced for oxygen tensions larger than 21%. In addition, the degree of the shift is best expressed at 38.5°c.

G. Combined Effects of X-irradiation, Oxygen Tension and Magnetic Fields upon Wing Anomaly Induction:

The experiments described in Section II,B,4, were designed to test the interaction between radiation (serving as a source of new oxygen toxicity centers), post-irradiation ambient gas composition and magnetic fields. Figure 11 and Table VII describe the results obtained. It is evident that the magnetic field did not have significant effect for oxygen tensions up to 21%. However, for higher oxygen tension, a shift to the left in this part of the curve is obtained. In other words, to induce a given amount of wing amonaly pupae exposed to magnetic fields require less oxygen than their corresponding controls. As a working hypothesis, it can be said that when dealing with higher oxygen tensions, the magnetic field increased the "effective" oxygen concentration.

The data also suggest that in this biological system there is a post-irradiation oxygen effect and that magnetic fields modify such effect. An analysis of these observations is given in Table VIII. By comparing the incidence of wing anomaly at a post-irradiation ambient composition of 40% oxygen with that resulting from post-irradiation incubation in air, we find that the ratio is 1.6. This ratio for the non-irradiated control is 1.2. The difference reflects the amplification due to the oxygen effect. When magnetic fields constitute an added parameter to radiation and oxygen, the ratio is found to be 2.8.

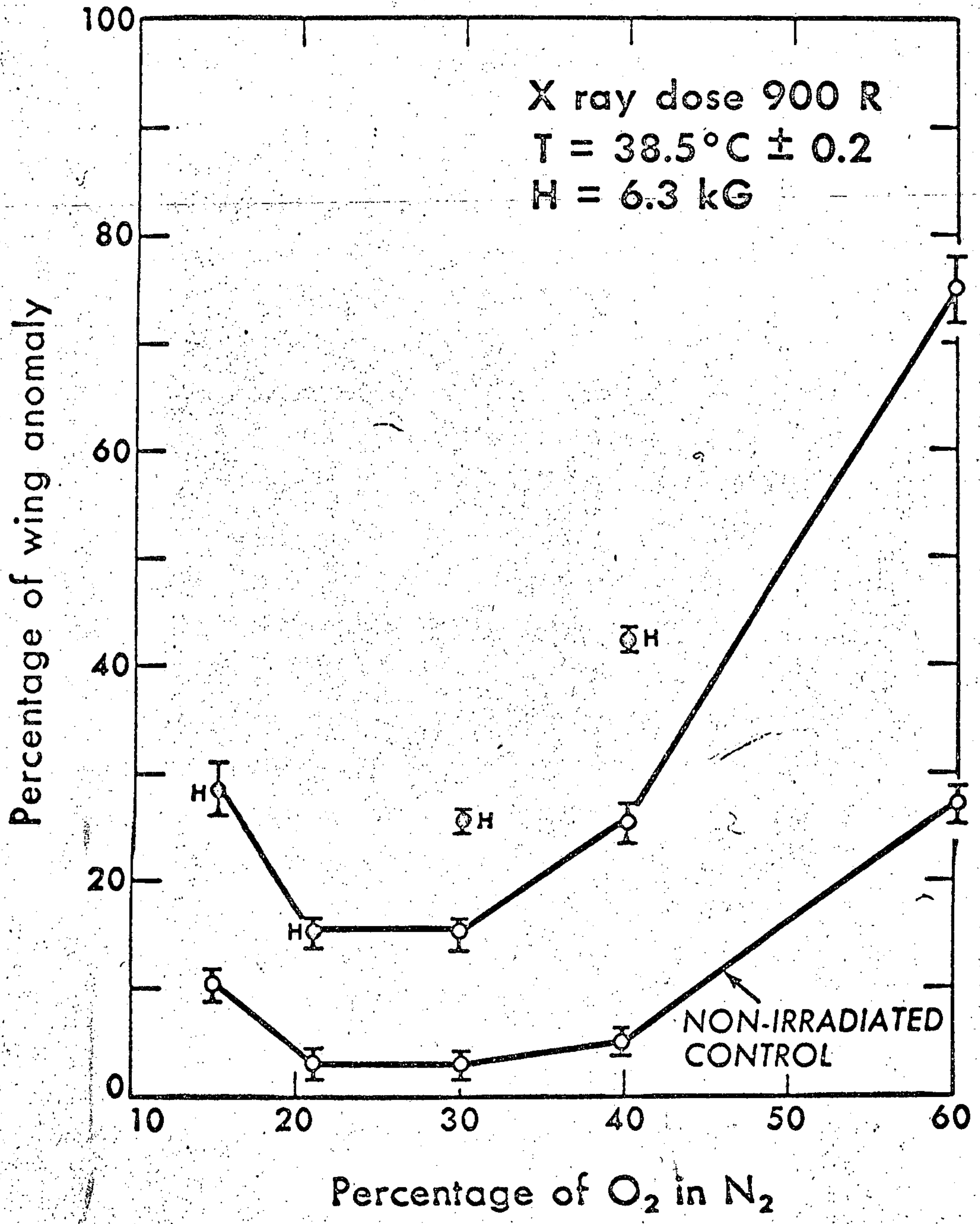


Figure 11.

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TABLE VII: Combined Effects of Magnetic Fields*, X-irradiation**, Ambient Gas Composition, and Incubation at 38.5°C ± 0.2 upon Wing Anomaly

Experiment	Number of Animals per Column	Percent Wing Anomaly at Gas Composition of							
		15% Oxygen		21% Oxygen		30% Oxygen		40% Oxygen	
		C	H	C	H	C	H	C	H
1	300	32.3±2.7	31.3±2.6	18.0±2.2	20.3±2.3	12.3±1.9	18.7±1.9	30.3±2.6	49.7±2.9
2	300	27.0±2.8	37.7±2.8	12.0±1.9	19.0±2.3	15.0±2.1	20.3±2.3	27.7±2.6	43.7±2.9
3	300	32.3±2.7	28.7±2.6	12.3±1.9	11.7±1.9	21.0±2.3	32.3±2.7	30.7±2.6	49.3±2.9
4	300	29.3±2.6	28.3±2.6	9.7±1.7	12.0±1.9	17.7±2.2	36.3±2.8	35.0±2.7	51.0±2.9
5	300	20.7±2.3	19.7±2.3	21.0±2.8	18.3±2.2	18.0±2.2	30.0±2.6	28.7±2.6	41.3±2.8
6	300	30.3±2.6	32.0±2.7	14.0±2.0	15.7±2.1	11.0±1.8	16.7±2.1	17.3±2.1	35.3±2.8
7	300	25.7±2.5	25.0±2.5	13.7±2.0	12.3±1.9	13.0±1.9	18.3±2.1	21.0±2.3	36.7±2.8
8	300	31.7±2.6	28.0±2.6	17.7±2.2	15.0±2.1	19.3±2.3	31.3±2.6	21.3±2.3	39.3±2.8
9	300	24.0±2.5	21.3±2.3	19.0±2.3	19.0±2.3	18.0±2.2	27.0±2.6	18.0±2.1	34.7±2.8
10	300	22.3±2.4	27.3±2.6	16.0±2.1	13.3±2.0	14.0±2.0	18.7±1.9	30.7±2.6	44.0±2.9
Mean of all experiments ± s.d. of the mean		28.6±2.6	27.9±2.6	15.3±2.1	15.6±2.1	15.9±2.1	25.5±2.4	26.0±2.4	42.5±2.8

* Field Intensity: 6.3 kG

** Radiation Dose: 900 R

TABLE VIII

Analysis of the Influence of Magnetic Fields upon Post-Irradiation Oxygen Effect

Ratio of Wing Anomaly	Control	Irradiated (900 R)	Irradiated + 6.3 kG
$\frac{30\%\ \text{Oxygen}}{21\%\ \text{Oxygen}}$	1.0	1.0	1.7
$\frac{40\%\ \text{Oxygen}}{21\%\ \text{Oxygen}}$	1.2	1.6	2.8

H. Additional Morphological Observations:

1. Effects on Longevity of Adults:

The adult populations of both the experimental and the control were kept, after scoring for data collection, in flour at 30°C. for further observations. Abnormal adults from magneto-treated groups suffered much less from latent lethality than those of the control groups. The period of time at the end of which lethality was scored was 4 months.

2. Effects on Pigmentation and General Health of Adults:

A phenomenon which persisted throughout the experimental works reported earlier was the more complete pigmentation and healthy appearance of the adults from the magneto-treated groups in comparison with their controls.

3. Effects on the Severity of the Wing Anomaly Induced by Radiation:

An interesting and consistent observation was that the intensity of the radiation-induced damage, based upon the degree of displacement of both membraneous and external wings, of the deformed portion of the magneto-treated group was morphologically lesser in magnitude than that of the control.

IV. DISCUSSION

Before attempting to interpret the results described in the previous section, a discussion of the experimental criteria in relation to the developmental pathway of the pupa is appropriate.

We shall represent, in a schematic manner, the metamorphosis of a pupa by an arrow terminating with the formation of a normal adult. Moreover, we shall adopt the thesis that the experimental criteria described above are not the outcome of a different direction or directions in the arrow of metamorphosis; rather, they represent arrest, at different points, of the progress along that arrow.

This conclusion was achieved after making and carefully studying time-lapse movies of the development of Tribolium pupae. (Amer and Camp(53)) Particularly, we believe that the wing anomaly is a step short of achieving the goal of development, since morphologically the movement of the wings in the pupa molting into adult (a process which takes about 3 minutes at room temperature) is a smooth and continuous one: from the pupal ventral position to a lateral one then to a final dorsal position. Adults which develop wing anomaly fail to complete this movement to its end; i.e., the full translocation of the wings to the dorsal position.

To link these morphological observations to the endocrinological control over the processes of growth, differentiation and development one should bear in mind the counter-acting role of ecdysone and juvenile hormone. As we mentioned earlier in Section I, juvenile hormone is a conservative agent, i.e., it induces pupa-pupa type molt rather than allowing the natural progress of growth and differentiation leading to pupa-adult type molt. For such a path to occur, ecdysone has to be present and the juvenile hormone has to vanish. Williams(54,55) reported that the injection of a small dose of juvenile hormone at that stage would lead to the formation of a strange product possessing a combination of adult duct possessing a combination of adult and pupal characteristics. A larger dose injected at the outset of the adult development leads to the molting of the pupa into a second pupal stage. It has been reported as early as 1933(56) that the irradiation of Calliphora larvae resulted in the molting of a pupa which exhibited a mixture of larval and pupal tissue.

In the light of the endocronological background presented above, one may assume that the process of irradiation distorted the delicate balance between the two hormones necessary for normal development. This distortion could be accomplished by one or more of the following possibilities: the full or partial blocking of the natural inactivation process of the juvenile hormone, the destruction of the effectiveness of ecdysone, the interference with the release of the brain hormone necessary for the activation of the prothoracic gland which secretes ecdysone, and/or the destruction of the effectiveness of the brain hormone itself.

In analyzing the results described in Section III, several alternatives offer themselves. One may assume that during the process of metamorphosis, there occurs a natural change in a certain crucial molecule or molecules from a diamagnetic state to a paramagnetic one. This change enables the applied external field to interact with that molecule. Magnetic susceptibility studies with biochemical compounds showed the existence of such a change in the magnetic state. Since we are dealing predominantly with organic molecules and from evidence discussed in Section I, we may hypothesize that at the molecular level during some stage of the developmental process, free radicals are formed. The effect of the magnetic fields on such molecules would be an orienting one, and perhaps in addition the free radicals might become decoupled to varying degrees from magnetic reorienting forces in their immediate vicinity.

Free radicals possess their magnetic properties because of one unpaired electron. Thus the spin S attributed to such a radical is 1/2. The orbital angular momentum is so strongly quenched that the spectroscopic splitting factor, the g-value, is virtually the same as g_s (= 2.0023). In an external field H, the energy levels are those of a magnetic dipole of moment $g\mu_B S$ with (2S + 1) allowed orientations is given by

$$W_{S_z} = g\mu_B S_z H$$

where μ_B is the Bohr magneton and S_z is the projection of S upon H.

Adhering to a Curie law dependence, and since, in eventuality, H is almost always restricted to values such that

$$g\mu_B H \ll kT$$

then the volume magnetic susceptibility κ for free radicals is given by

$$\kappa = N\mu_B^2\, 4S(S+1)/3kT$$

where N is the number of paramagnetic centers per unit volume.

From the argument given above, thermal energy imposes a limit as to the degree of interaction between the magnetic field and the paramagnetic centers. Consequently, any alteration in the rate of interaction between these oriented centers and other molecules or other paramagnetic centers will be dependent upon the ratio of the magnetic interaction with the field and the energy of thermal agitation. Even in restricting our analysis to such idealized arguments, the data we obtained indeed conforms with that thermodynamical limitation. The results described in Section III, C, show that the effect of the field may have been equivalent to a "cooling" effect of approximately 1°C/8000 gauss. This is in the same order of magnitude as the ratio $g\mu_B H/KT$ where T is the amount of equivalent "cooling", i. e., 1°C. However, in examining a biological system, it is evident that an oversimplified model of free non-interacting magnetic centers cannot adequately represent such a complex structure. Consequently, the 1/T dependence of free radical contribution to magnetic susceptibility will not be linear. Arguments supporting this expected departure from the Curie Law are as follows. A fixed concentration of free radicals should obey the Curie Law. But the concentration of free radical intermediates in a biologically active system is a function of temperature, which is the resultant of temperature dependence of several rate constants, and the concentration of the reactants. Brill(57) concludes that it is "almost certain that the free radical contributions to the magnetic susceptibility will not have a dependence which is linear with 1/T."

Another point which has to be taken into consideration is the geometrical nature of important biological structures which possess some periodicity or quasi-periodicity. Perhaps such ordered structures with localized atoms in space pose some restrictions on the efficiency of thermal agitation. Experimental work has shown that the EPR signals from irradiated single crystals of organic and biochemical compounds are different from those obtained with powder forms.(58) The difference is due to the orientation of the radical in specific directions, which correspond to the orderly structure of the crystal itself.

Another departure from the Curie Law is found in the Van Vleck theory of magnetic susceptibility.(59) In his calculations, he showed that in cases where the energy separation between the ground and the excited states is much larger than kT, a term for susceptibility which is temperature independent, is obtained and is known as Van Vleck paramagnetism.

Molecular oxygen itself is a free radical, and free radicals usually interact strongly with other free radicals. The results we obtained with oxygen are highly suggestive in this regard. All our data can be explained by assuming that due to the orienting and decoupling influence, the magnetic field has accelerated the interaction of some radicals with oxygen. Thus, at low oxygen tensions, the action of the field is to raise the interaction probability; therefore the organisms can live and develop at oxygen concentration lower than normal. At high concentrations, where oxygen is toxic, the rate of interaction of some free radical with oxygen has been accelerated, consequently oxygen has become more toxic.

This same line of reasoning enables one to explain, qualitatively, the data obtained from the irradiation experiments: as discussed in Section I, ionizing radiation induces the formation of free radicals in tissue, and the evidence is rapidly mounting to implicate some of these in the radiation lethality process. Here, the orienting effect of the magnetic field might either increase the need for oxygen for normal development to take place; or, alternatively, accelerate the rate of decay of free radicals produced by radiation. At high fields ($>$8kG) however, the probability of interaction with oxygen has increased to the domain of oxygen toxicity. Thus explaining the rise seen in Figure 6 at field intensity of 9.7 kG.

In the results described in Section III, D, and graphed in Figure 13, the lack of any effect of magnetic fields at oxygen tensions of 21% or less could be explained as follows. Powers et. al.(60) divided radiation damage into two main components: one is oxygen-dependent and the other is independent of oxygen. We believe that the observed absence of magnetic field effects at lower oxygen tensions could be explained by assuming that at low oxygen concentrations we are dealing with the oxygen-independent component of radiation damage. Therefore, magnetic fields have no potential in altering that damage; while at higher oxygen concentrations, the oxygen-dependent component participates in the damage process, and applied magnetic fields, via their interaction with the biradical oxygen, affect the kinetics of the damage process leading to the observed effect.

We have observed that at high oxygen concentrations (80% or more), development is blocked and the pupae suffer pupal death. This arrest in developmental progress resembles the effect of lack of ecdysone, or the persistence of large amounts of juvenile hormone. Although the exact chemical structure of ecdysone has yet to be determined, it has been found to be a steroid with the empirical formula $C_{27}H_{44}O_6$. Karlson et. al.(61) found that cholesterol is a precursor of ecdysone. Gilbert(62) suggests that the brain hormone is a sterol and that possibly juvenile hormone is a sterol precursor. Then the three important hormones in insect development are substances with similar and related chemical properties. These findings point out the universal importance of steroidal compounds in regulating growth and development not only in the invertebrates but in the vertebrates as well.

Because of the existence of unsaturated bonds in these hormones, and from our results involving oxygen, we have formed the hypothesis that the oxygen toxicity in developing insects may at least in part be due to oxidation of the hormonal system to some biologically inactive state. The influence of magnetic fields is to enhance the degree of oxidation.

It is known from the work of The Svedberg(63) that magnetic fields influence the electric conductivity of liquid crystals which are substances exhibiting anisotropy in magnetic and electric fields, and whose chemical structure is closely related to the hormones involved in the process of development. Svedberg explained the observed change in electric conductivity as due to changes in viscosity brought about by the external field yielding a preferential orientation of the axes of the molecules parallel to the lines of force. Such findings add another dimension to the possible ways in which magnetic fields may interact with those biological systems where cholesteric substances are of prime physiological importance.

ADDENDUM

While writing this paper, the author embarked on a set of experiments utilizing a liquid crystal material known commercially as Spectratherm (a mixture of cholesteryl benzoate and cholesteryl nonanoate). This material, like other cholesteric liquid crystals, has a unique molecular geometry: the molecules are arranged in layers. These layers are very thin with the long axes of the molecules parallel to the plane of the layers. Because of the molecular structure, the long axes of the molecules in each layer are slightly displaced from the corresponding direction in the adjacent layers; the overall displacement traces out a helical path. These substances, though colorless as liquids, go through a series of bright colors when cooled through their liquid crystal phase. In that phase they first assume a violet color, then blue, then green, then yellow, then red and, finally, colorless again. Cholesteric substances scatter light selectively rather than absorb it. In our work we chose a substance which has a range of coloration between 20° and 23°C. By maintaining a constant temperature, we found that the magnetic fields (6.3 kG) changed the blue color to orange. It is known(64,65,66) that one obtains such response by either one of two ways: 1.) Lowering the temperature; or 2.) Introducing oxidizing agents in the environment of the cholesteric material.

Although our results are of a preliminary nature, their implication in the hypotheses we offered earlier to explain our findings with Tribolium may be significant.

V. SUMMARY

From the results discussed above the following conclusions can be drawn:

1. When Tribolium pupae were allowed to develop in magnetic fields, in air, and at 38°C, X-ray induced wing anomaly decreased with increasing field intensity up to 8 kG. This "protective" effect is less expressed at higher fields.

2. At higher incubation temperatures (39.5°C), magnetic fields reduced the incidence frequency of heat induced molting failures.

3. The effect of a field of 6.3 kG on developing pupae at various oxygen tensions is to increase the interaction with oxygen, thus decreasing effects due to anoxia in low oxygen tension environments, while increasing oxygen toxicity at high oxygen tensions. When ionizing radiation is an added parameter, the field has no influence at low oxygen tensions; on the other hand, for high oxygen tension the damage is enhanced.

4. Since the hormones governing development and growth in this biological system are either steroids or precursors of steroids, we suggest that oxidation of steroids may play a role in the oxygen toxicity, and that magnetic fields may play an important part in influencing the rate of oxidation of unsaturated steroids.

5. A possible molecular interpretation to our findings may be achieved from results we obtained working with cholesteric substances. Our preliminary findings support the hypothesis we presented above where magnetic fields afford a degree of "cooling" as well as enhancement of the rate of oxidation.

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