EFFECTS OF WEIGHTLESSNESS, GRAVITY COMPENSATION AND
RADIATION ON THE FLOUR BEETLE, TRIBOLIUM CONFUSUM*

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1. Overview

For a number of years the Donner Laboratory group has studied various aspects of the effects of physical environment on the flour beetle, Tribolium confusum. Initial radiobiological studies using low-energy accelerated heavy ions from the Berkeley MILAC allowed quantitation of the occurrence of a developmental abnormality -- wing malformation and localization of the sensitive region in pupae (1). It had been shown by J. Beck (2) that this abnormality in irradiated pupae was not identical with the well known genetic wing abnormalities studied by Sokoloff (3).

The wing abnormality is defined as: (1) Deformity of the external wings (the elytra), which appears as a slight, irregular warping, and dorsal and lateral displacement from the normal position over the membranous wings, and the dorsal lateral 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 (Figure 1). The joint action of radiation and temperature indicated a synergistic effect (4). It was shown, moreover, that radiation, temperature, and the presence of constant magnetic fields all altered the occurrence of wing abnormalities and normal development of the beetles (5). Ambient gas composition also had some important effects. All these experiments led to formulation of some general ideas on the effects of various environmental agents on organisms with homeostatic control (6).

Tribolium confusum was chosen as a test organism for determination of possible synergistic effects of radiation and space environment in the inertial flight of Biosatellite-II. This experiment was carried out mainly by Slater and Buckhold (7). The organisms subjected to weightlessness and radiation during the flight exhibited greater than

expected wing abnormalities. However, a postflight "vibration control" experiment produced anomalous results, and some doubt remained with respect to assigning weightlessness as the sole cause of the increased wing abnormalities.

This paper is an account of experiments performed on the interaction of gravity compensation, radiation, and Tribolium development. It was found that gravity compensation together with radiation did not cause more wing abnormalities than those caused by radiation alone (beetles irradiated stationary at 1g). However, radiation and gravity compensation plus high temperature did cause an increased percentage of wing abnormalities. Two different conclusions are possible:

1. We could assume that gravity compensation is equivalent to weightlessness; then there may exist some other environmental factors in space flight, perhaps vibration and cosmic rays, that interact to produce the observed results.
2. We cannot be sure that weightlessness and gravity compensation are equivalent; in fact, in this paper we present a physical analysis to show they are different. We then conclude that further light on this subject can be obtained only if additional future space-flight experiments take place with Tribolium. We have proposed such experiments, in which weightlessness is compared to artificially-created gravity fields (in a centrifuge) on board the spaceship.

With the increased knowledge made available on Tribolium through heavy-ion irradiation and additional clinostat studies, a series of tests could be conducted in space with additional biological endpoints. A prolonged flight involving the entire life cycle or perhaps several reproductive generations of Tribolium would be of great interest, since our preliminary data indicate that beetles developed under gravity com-

pensation from egg stage may be more sensitive to radiation and to other space environment factors.

II. Introduction

Potential effects of the space environment on living organisms have attracted scientists' attention since the beginning of the space program in this country. Our group has actively participated in space radiobiology research and conducted experiments both on the ground and in space. In 1961, we flew plant seeds to altitudes of 85,000 to 125,000 feet in balloons for a 48-hour period in order to explore the responses of higher organisms to the space environment (8). After these seeds were successfully recovered and planted, some developmental abnormalities were observed in the flight group, suggesting detrimental effects of the space environment. In 1966, an opportunity was provided by NASA for us to participate in the Biosatellite-II experiment program. Tribolium confusum pupae were chosen as the experimental biological system to be subjected to the stresses of ionizing radiation and gravity-free environment for four main reasons:

1. Experiments were planned to assess the effects of weightlessness over a relatively short time period, i.e., 72 planned orbital hours. Following theoretical calculations by Pollard, it was believed that small unicellular organisms might not show effects of weightlessness due to their small size. A developing multicellular organism of relatively large size and with many simultaneous ongoing life processes was thought, therefore, to be a more sensitive object.
2. Hormonal control in development of the pupal stage is well established; moreover, the relatively small number of hormones involved in the developmental process makes such a system attractive.
3. Extensive radiobiological data are available on Tribolium.

4. The life support system of this organism is exceedingly simple: these beetles can develop and exist from egg stage to adult in dry flour. Eventually population genetics studies in space should be possible using this organism.

The goal for this experiment was to study effects of weightlessness only, compared with the synergistic effects of weightlessness and radiation on developing organisms. A total of 720 Tribolium pupae were orbited in the biosatellite, half in a module in the presence of an Sr^{85} source and half shielded from it. Upon return of the beetles flown on the satellite, three separate studies were initiated. First, eclosion time was measured in order to determine the length of the pupal period. Second, the percentage of wing abnormalities was determined. Finally, experimental beetles were crossed with incubator controls in order to ascertain the extent of the genetic effect in terms of dominant lethality. It was found that, in beetles which were both preirradiated and chronically irradiated in flight, there was a significant 50% increase in wing abnormalities compared with the ground control; that eclosion might have been slightly delayed as a result of the flight; and that dominant lethality was somewhat increased in the offspring of flight females irradiated with gamma rays in space (7).

These experimental results point to the synergistic effect of weightlessness on radiation damage. An inhibition or suppression of repair system in cells under weightlessness was suggested as one of the possible mechanisms involved in the observed synergistic effect.

In order to have a further understanding of the effect of weightlessness on the development of higher organisms without conducting experiments in space, we initiated the present clinostat work. In this report, the clinostat

facilities will be described; theoretical analysis of compensated gravity will be presented; some experimental results will be analyzed; and a future research plan will be suggested.

III. Theoretical Analysis of Gravireceptor Dynamics in Compensated Gravity.

In order to provide appropriate theoretical guidelines for clinostat investigation, a member of our group, Dr. Ira Silver, developed a model for theoretical analysis of the dynamics of a discrete geotropic sensor subject to rotation-induced gravity compensation. Since this theoretical work has been submitted for publication in the Journal of Theoretical Biology, a summary only of the theory will be presented here.

In a cell, a discrete subcellular component, or organelle, is subjected to the following forces:

$$|G| = \text{magnitude of the gravitational force} \sim (1 - m_F/m_T)g = K_1;$$

$$|C| = \text{magnitude of the centrifugal force} \sim (1 - m_F/m_T)R\omega = K_2;$$

$$\bar{V} = \text{viscous force} = -6\pi\eta r_T \bar{V} = -K_3 m_T \bar{V} \quad (\text{Stoke's law});$$

where m_T is the mass of the geotropic receptor,
 m_F is the mass of the displaced cellular fluid,
 g is the acceleration of gravity,
 R is the clinostat radius of rotation, and
 $\bar{V}$ is the instantaneous velocity.

The consequent differential equation for Newton's law of motion of the georeceptor during a 360° rotation of the clinostat will be:

$$\ddot{y} = K_1 \sin(\omega t) + K_2 - K_3 \dot{y}; \quad (1)$$

$$\ddot{x} = K_1 \cos(\omega t) - K_3 \dot{x}.$$

Based on the initial conditions that

$$x = \dot{x} = 0, \quad y = (K_2/K_3) - (K_1/K_3)(1/\omega) \text{ and } \dot{y} = 0, \text{ at } t = 0,$$

and assuming an isothermal and homogeneous cytoplasm, equations (1) and (2)

will result in:

$$y(t) = \left[\left(\frac{K_2(\omega^2 + K_3^2)}{K_1 K_3^2} - \frac{\omega}{K_3} \right) e^{-K_3 t} - \frac{K_3}{\omega} \cos(\omega t) - \sin(\omega t) \right] \frac{K_1}{(\omega^2 + K_3^2)} + \frac{K_2 t}{K_3}; \quad (3)$$

$$x(t) = \left[e^{-K_3 t} - \cos(\omega t) \right] \frac{K_1}{(\omega^2 + K_3^2)}. \quad (4)$$

For cellular components, $K_3/\omega \sim \eta r_F/m_F n \gg 1$. The harmonic contribution to the particular solution of $y(t)$ will be:

$$y(t_m) = K_1/K_3^2 \{ (-K_3/\omega) \cos(\omega/K_3 + \pi) - \sin(\omega/K_3 + \pi) \} = K_1/K_3 \omega.$$

Therefore, maximum amplitude of the harmonic motion is proportional to $1/\omega$.

It is evident that gravity compensation is more significant at higher speeds as the amplitude of the harmonic motion decreases. The rate of displacement due to centrifugal force, however, increases with increasing rotation rate. The dynamics of a discrete and free-moving geotropic receptor subject to clinostat rotation results in two opposing tendencies. Circular motion, *i.e.*, a short-range effect, is induced with an amplitude (v_1/ω) , and with a consequent deviation from thermal motion $(v_e^{(A/\omega)})$, where

$$A = (m_F - m_F)g(2K_1/K_3)/kT.$$

There is also induced a unidirectional motion, *i.e.*, long-range effect $(v\omega^2)$. The efficacy, E , of the attempted gravity compensation is thus a function of these two factors and can be written as:

$$E = (e^{(A/\omega)})^a (v\omega^2)^b,$$

where a and b are "weighing factors", > 0 .

For a given system, the "best" rotation rate exists when E is maximized. Therefore,

$$\frac{\partial E}{\partial \omega} = 0, \text{ and } \omega = \left(\frac{A}{2}\right)^{\frac{a}{b}}.$$

Using experimental data of Rutten-Peckelharing (1910), it was found that a/b is 3 at optimum rotation speed. The best rotation rate for a given biological system can, therefore, be expressed as:

$$\omega = \frac{1}{2\pi} \cdot \frac{(m_F - m_F)^2 g^2}{\eta r_F kT},$$

where K is Boltzmann's constant,
 T is the absolute temperature, and
 η is the viscosity of cytoplasm.

IV. Clinostat Facilities

In order to be able simultaneously to run gravity compensation experiments with small multicellular organisms and to expose these organisms to radiation, Dr. Ira Silver of our group built a pair of identical 1m clinostats. The "radiation clinostat" was in a shielded room adjacent to a Cs¹³⁷ gamma-ray source that could deliver continuous gamma-rays at a dose rate up to 50 rads per minute. The clinostat could be adjusted to have its axis horizontal, vertical or at some angle in between. Rotational speeds could be varied from 2 rpm to 128 rpm. Air temperature and humidity control were provided, and vibration and sound levels were minimized. The "control clinostat" was mounted in a second room, identical to the "radiation clinostat" but without radiation source. Some details of the construction and features of the clinostats are given here.

A. General Information

To determine the synergistic effect of gravity compensation and radiation on biological systems at ground level, a clinostat equipped with an irradiation source is necessary. Selected for their simplicity, two "1m" clinostats were designed and built in our laboratory under a joint program of the NASA Bioscience Division (Purchase Order #W12-792 Task 6) and the Energy Research and Development Administration (ERDA), (formerly the AEC). One clinostat is used with a cesium irradiation source, and the other serves as a non-irradiation control.^{*} The clinostat irradiation complex is a self-contained unit housed in Building 74-B at the Lawrence Berkeley Laboratory. A schematic of the floor plan is shown in Figure 2. Both the control and the irradiator rooms are temperature-controlled and soundproofed. In addition, the latter room is shielded by

^{*} In the course of designing the clinostat, we received valuable advice from the late Dr. Solon Gordon of the Argonne National Laboratory.

concrete, as indicated, and has a steel door (6.5 ft. high x 4.5 ft. wide x 1 ft. thick) opening to the outside. Electrical wiring, water cooling system and air ducts lead from the laboratory area to both rooms via ceiling and floor trenches. An interlock system isolates the irradiator room from the rest of the complex during irradiation. All control operations are handled in the laboratory area. The air conditioner, temperature and humidity recorders, water cooling pump, and irradiator control console are all along the walls common to both the laboratory and the clinostat rooms (Figure 3).

B. Clinostat

The clinostats are identical, measuring 17 inches high, 17 inches broad, and 7 inches deep (excluding attached specimen holders), and each weighs approximately 100 pounds. Each consists of nine parallel, horizontal, ventilated, rotating shafts, capable of operating at speeds of 2 to 28 rpm. Eight of these shafts are evenly spaced on the circumference of a circle 9 inches in diameter, at the center of which is located the ninth shaft, as shown in Figure 4. Simultaneous controls are provided by a vertical shaft rotating at the same speed as the horizontal ones, as well as by four horizontal stationary studs. Each clinostat is run by two, three-speed, synchronous electric motors enclosed in water jackets. The motors are placed in parallel and are situated on diagonally opposite sides of the clinostat to ensure proper balance. Motion is transmitted to the shafts from the motors via capstans engaging a flywheel, which then moves an endless Mylar belt passing over one of two sets of shaft pulleys. One set of pulleys sets the shaft speeds at 8, 16, 32, 64 or 128 rpm; the other set reduces said speeds by a factor of four. In fact, the shaft speeds can be further reduced by changing the relative diameters of the flywheel and capstans.

Reference to gravity is made by means of bubble levels. When the clinostat is tilted, a circular bubble level is used to adjust the shafts to the vertical via a mirror arrangement. Manual precision leveling screws

permit reference adjustment. The shafts are parallel to each other within 1 part in 10,000, and the vertical shaft is perpendicular to the horizontal to 1 part in 1,000. The sag and vibration of a typical specimen holder on the shaft is negligible.

The clinostats in chambers have been lined with heavy lead foam padding to attenuate outside noise as well as dampen internal noise. The noise level in the clinostat locale has been measured and the noise generated by each clinostat found to average 47 decibels, varying about 4 db in the speed range 8 to 128 rpm. The effectiveness of the clinostats in producing compensated gravity was studied using broad bean roots, and we found that by this method the gravity compensation produced by the clinostat was equivalent to $10^{-3}g$ or less. In addition, a physical measurement on sensitivity of changing the leveling of spindles in the clinostat was made, and it showed that a change in height of 0.25 mm of clinostat could be easily detected with the built-in level. We believe that our clinostat compensates to better than $10^{-3}g$, perhaps $10^{-4}g$.

C. Radiation Source

Irradiation capabilities are provided by a nominal strength 1000-curie, cesium-137 source (1042 Ci on 8/28/65). The source has been installed on a double-track crane with a five-ton lift capacity. The consequent irradiator system is contained within a concrete-shielded room. The crane has horizontal and vertical movement at an electrically controlled speed of 15 inches/min. In addition, the source can be electrically moved through 90° from a horizontal irradiation position (Figure 5) to a vertical position (Figure 6). Dose calibrations show that maximum irradiation rate for the clinostat is about 50 roentgen/min. Proper positioning and shielding can reduce this flux to any desired rate.

D. Temperature, humidity and gas control

Air, supplied by an Aminco-Aire air conditioner, passes in parallel through both environmental chambers in which the clinostats are housed. Flow is through a closed system with air being bled from the main duct via butterfly valves into each of the chambers. Air passes through flexible tubing into a baffle system on the side of the chamber and is thus diffused into the chamber, prior to exhausting to the ambient. Air filters are placed in each line, immediately after the bleed-off point. In order to maintain a sterile environment, an ultraviolet germicidal lamp is installed at the air conditioner outlet. Resistance heaters, installed in each line, just beyond the air filter, supplement the heat supplied by the air conditioner. In addition, these heaters are used in conjunction with two Wheelco temperature regulators to maintain constant temperature in each clinostat. These regulators work on a thermocouple sensor input. In the range of 20 to 32°C, variations less than 0.5°C can be obtained between the sensor locations at each clinostat. Temperatures up to 40°C are available with this system. A concurrent relative humidity range of 20 - 80% is available from the air conditioner.

Provision has been made to allow any desired "atmosphere" to pass over the test specimens. The shafts on the clinostat are hollow and tubes attached to them through the clinostat face plate pass out of the respective chambers to any desired gas supply. In fact, one tube services two horizontal shafts, so that four separate atmospheres can be employed simultaneously.

V. Experimental results

A. Experimental program of *Tribolium confusum* using the radiation clinostat.

The main purpose of the experiments with the clinostat was to determine whether or not gravity compensation leads to effects similar to those found in space flight. For this purpose, developing pupae were used, and we found similar, though less significant synergism with radiation than under space-flight conditions.

We then generalized the inquiry to other stages of *Tribolium* development. This was done to gain a greater insight into the effects of gravity compensation. Fertilized eggs, larvae, pupae and adults were eventually tested. The data obtained can be used in connection with future prolonged space flights during which the entire life cycle and perhaps several generations of *Tribolium* will be under weightlessness.

B. Development of Pupae.

1. Formation of adults from pupae with gravity compensation.

Following completion of the clinostats, experiments were conducted to investigate the response of young Tribolium pupae under compensated gravity. Pupae collected within 6 hours at 30°C were incubated for another 18 hours at 30°C before they were placed in pupae holders. The clinostat specimen holder with pupae holder is shown in Figure 7. More than 60 holes with the same size as Tribolium pupae have been drilled and spaced evenly in the plastic pupae disc, and four discs together can be assembled with two brass screws. About 50 pupae can be put in holes of each plastic disc; this prevents pupae from tumbling during rotation. Spaces are placed between discs so that air can circulate through the pupae holder.

Three experiments were performed to determine the ability of pupae to develop into normal adults under gravity compensation; results are tabulated in Table 1. Pupae in pupae holders were kept in the clinostat for 4 days, and the number of normal adults were checked within 3 days after pupae were removed from the clinostat. At 34°C it was found that pupae showed no difficulty in developing into adults under compensated gravity: there was no significant difference in number of normal adults formed between horizontal stationary (S_{1g} : 0rpm)* and horizontal rotated (R_{1g} : 4rpm)** group.

2. Wing development of gamma-irradiated pupae under compensated gravity.

Results obtained from Tribolium pupae experiments on the Biosatellite-

II flight suggest that the radiosensitivity of pupae might be increased

*Horizontal stationary (S_{1g} : 0rpm): pupae were not rotated and were kept in a position perpendicular to the direction of gravitational force.

**Horizontal rotated (R_{1g} : 4rpm): pupae were rotated at 4rpm with the pupae holder perpendicular to the gravitational force.

under conditions of weightlessness. Accordingly, a series of experiments were done in the hope that this exciting result could be repeated here using the clinostat. Because of the low dose rate of the cesium-irradiator, rapid increase in radiosensitivity with pupal age, and the krad dose range needed to induce wing deformities, pupae were irradiated in the clinostat at 25°C. Four different doses, ranging from 2600 to 3650 rads, were given to young pupae (24 hours old at 30°C at the time of irradiation); experimental results are shown in Table 2. Except at the highest dose (3650 rads), no significant difference in percentage of wing deformity between horizontal stationary (S_{1g} : 0rpm) and horizontal rotated groups (R_{1g} : 4rpm) was observed.

3. Gamma-irradiated pupae under compensated gravity and high temperature.

Since pupae receiving a high lethal dose (i.e. 3650 rads) exhibited a higher incidence of wing malformation under gravity compensation, experiments were designed to study the radiation response of pupae under compensated gravity and high temperature. A total of 4362 pupae were used in the first series of experiments; results obtained are shown in Table 3. At 39°C, pupae irradiated under gravity compensation all had a higher percentage of wing abnormalities. Statistical analysis of these data shows that pupae receiving gamma-irradiation of 2194 rads and 2947 rads, respectively, at a dose rate of 2 Rads/min under gravity compensation developed significantly more wing deformities than those irradiated at 1 g.

In order to further verify this finding, another series of experiments were initiated. In the second series of experiments, a total of 4117 pupae were used, and some pupae were rotated vertically (R_{1g} : 4rpm)*. Results of this second series of experiments are summarized in Table 4. Again, consistently higher wing abnormalities in horizontal rotated and

*Vertical rotated (R_{1g} : 4rpm): direction of the pupae holder is parallel to the direction of gravitational force and rotated at 4rpm.

irradiated groups (R_{13} : 4rpm) was found. Pupae irradiated with 2700 rads under compensated gravity showed a highly significant decrease in normal adult development. No significant difference in wing development was noticed in vertical rotated and irradiated groups when compared with those not rotated; this seems to indicate that the observed increase in radiosensitivity of pupae under gravity compensation is not due to centrifugal force.

4. Effect of low temperature and gravity compensation on radiosensitivity.

The fact that radiosensitivity of pupae is enhanced by compensated gravity at high temperatures (38° to 39°C) points to the possibility that a similar synergistic effect can be observed at a temperature lower than the optimum range for Tribolium development. Consequently, experiments were done exposing pupae to 3°C for various periods before they were placed in the clinostat and gamma-irradiated. Results showed that there was some increase in radiosensitivity of pupae which were exposed to 3°C for 1 to 3 days and which received gamma-irradiation of 3500 rads and were rotated at 16 rpm in the clinostat (see Figure 8). When pupae were irradiated with a lower dose, i.e. 2000 rads, however, no change of radiosensitivity in pupae due to gravity compensation was found, although they were exposed to 3°C for 1 to 3 days before irradiation. Without irradiation, pupae exposed to 3°C up to 3 days and rotated at 16 rpm for 4 days at 31°C in the clinostat yielded 98 to 100% normal adults, values no different from incubator controls.

Later, a more systematic approach in determining the enhancement effect of low temperature (3°C) and compensated gravity on the radiosensitivity of pupae was made. Pupae were exposed to 3°C for 3 days and then gamma-irradiated with various doses under gravity compensation.

Results are shown in Figure 9. The slope of dose-response curve for pupae exposed to 3°C appears to be steeper than that for pupae not exposed to temperature, suggesting an increase of radiosensitivity.

C. Embryonic Development of Eggs.

Tribolium belong to the holometabolous group; these beetles have four definite developmental stages, i.e., egg, larval, pupal, and adult stages, as shown in Figure 10. In addition to young pupae, eggs were chosen as a second experimental system for the Biosatellite flight. Although eggs were not used at the time of flight, egg collecting techniques were developed. The following additional facts make the egg an attractive system for clinostat studies:

- 1) The size of egg is small: it is about 0.5mm long and about 0.1 mm wide. A large number of eggs can thus be put into a small container which can be rotated up to 100 rpm in the clinostat without generating a significant amount of centrifugal force.
- 2) The egg is a closed system and covered with a thin layer of flour. It is, therefore, easy to handle and needs no other medium for embryonic development.
- 3) The time needed for larval formation in the egg is about 4 to 5 days at 30°C, which is neither too short for giving eggs a lethal dose of radiation using the present dose rate of the clinostat, nor too long for getting experimental results within a practical time.
- 4) It is not impractical to collect more than a thousand eggs within a few hours. The age of eggs can thus be well controlled.

An extensive study on the effects of gravity compensation on biological development has, therefore, been performed with this system.

1. Compensated gravity only.

For centrolecithal eggs, e.g. Tribolium, superficial cleavage is

a characteristic phenomenon. The early divisions occur only in the surface layer of the egg and do not extend into the central yolk mass. During the cleavage, no polarity, i.e., orientation of cells in the egg with respect to gravitational force, can be seen. A longitudinal ventral furrow, however, starts to form at the gastrulation stage, and the cells of the dorsal surface divide and form the germ band. It is not known whether this polarity in the gastrulation stage is influenced by gravitational force, although some unusual abnormal types of development have been found in centrifuged Chironomus eggs (9).

The first series of clinostat experiments were designed to determine what role, if any, gravity plays in the embryonic development of Tribolium. Since there is no established method to measure the response time of eggs to the stress of gravity, a rotation speed of 32 rpm was chosen. In order to obtain good statistical results, more than 600 eggs were used for each group in each experiment. Eggs collected within 8 hours at 24°C were put in a clean glass capillary tube with I.D. 1.0 mm, which gives a centrifugal force less than $10^{-3}g$ at 32 rpm. A small piece of clean cotton was inserted at each end of the capillary tube, so that eggs were kept in place during rotation and air could circulate freely through the system. A complete clinostat Tribolium egg-holder system is shown in Figure 11. After eggs were rotated in the clinostat for 4 days at 34°C, they were transferred into clean glass dishes and kept incubated at 31°C. The hatchability of eggs was determined by counting the number of larvae that successfully came out of egg shells. Hatching was checked every day for 3 days after eggs were taken out of the clinostat.

Results of three separate experiments are shown in Table 5. No significant difference in hatchability between horizontal stationary ($S_{\perp g}$: 0 rpm)* and horizontal rotated ($R_{\perp g}$: 32 rpm)** eggs was found,

suggesting that compensated gravity does not inhibit the embryonic development of Tribolium eggs.

- * Horizontal stationary ($S_{\perp g}$: 0 rpm): eggs were not rotated and were kept in a position perpendicular to the direction of gravitational force.
- ** Horizontal rotated ($P_{\perp g}$: 32 rpm): eggs were rotated at 32 rpm with the capillary tube perpendicular to the gravitational force.

2. Effect of gravity compensation on X-irradiated eggs.

One of the interesting results obtained from the Biosatellite-II experiment is that radiation given to Tribolium pupae prior to space flight caused no significant change in wing deformity compared with ground controls. Similar experiments were done with the clinostat using Tribolium eggs. Three different rotation speeds, 8, 32, and 128 rpm, were chosen. The purpose of selecting a 16-fold increase in rotation speed (8 vs. 128 rpm) was to ensure that eggs were subjected to high gravity compensation; furthermore, this range is within the limitation of clinostat. Results of the series of experiments are shown in Figure 12. For each point on the curve, more than 400 eggs were used, and, for each dose and rotation speed, a separate experiment was done.

Eggs eight hours old kept at 24°C were collected from young adults. The eggs were X-irradiated, placed in glass capillary tubes, and rotated in the clinostat at various speeds for 4 days at 24°C. Hatchability of both X-irradiated and nonirradiated groups was checked after eggs were removed from the clinostat. No significant difference in hatchability between X-irradiated horizontal stationary and X-irradiated horizontal rotated eggs was observed. The slope of dose-response curves for 8, 32, and 128 rpm is about the same. One group of eggs was X-irradiated and rotated vertically at 8 rpm ($R_{\parallel g}$: 8 rpm)***, and hatchability was found to be no different from that of horizontal stationary or rotated groups. Results from these experiments therefore indicate that radiation given prior to gravity compensation causes no synergistic effect on the embryonic development of Tribolium eggs.

*** Vertical rotated ($R_{\parallel g}$: 8 rpm): direction of the egg holder is parallel to the direction of gravitational force.

3. Radiation and compensated gravity.

As the X-irradiated eggs showed no decrease in hatchability after exposure to gravity compensation for 4 days, another series of experiments were done to determine whether radiosensitivity can be increased during gravity compensation. Tribolium confusum eggs less than 8 hours old at 24°C were collected from young adults, and one group was horizontally rotated at 4 rpm while the other was kept stationary in the clinostat. After eggs were placed in the clinostat for about an hour, eggs were gamma-irradiated at 10 rads/min. Hatchabilities of eggs from six separate experiments are plotted in Figure 13. Radiosensitivity of eggs did not appear to change with compensated gravity.

In order to determine whether eggs of younger age are more sensitive to compensated gravity, eggs of 3, 4, and 6 hours old were collected and gamma-irradiated at 400 rads while exposed to gravity compensation for 4 days at 35°C. Comparing the horizontal rotated ($R_{\perp g}$: 4 rpm) with the horizontal stationary ($S_{\perp g}$: 0 rpm) of either gamma-irradiated or nonirradiated eggs, no difference in hatchability was observed, as shown in Figure 14.

Since it is very difficult to obtain the sufficient number of eggs to do a meaningful experiment when the egg collecting period is less than 3 hours, no experiment was done to determine whether eggs less than 3 hours old respond differently to compensated gravity. The present experimental results, however, suggest that embryonic development may not be sensitive to gravity compensation alone and that the radiosensitivity of young eggs developing at optimum temperature stays the same with compensated gravity.

4. Temperature, radiation, and gravity compensation.

One of the important environmental factors in space is temperature. Since temperature in the clinostat can be varied, an experiment was done

to study the radiosensitivity of eggs at high temperature with gravity compensation. After eggs were collected, they were put in the clinostat and gamma-irradiated in cycles (15 rads per 30 minutes at a dose rate of 10 rads/min) at 39°C. Results are presented in Table 6. Eggs not rotated and irradiated but kept at 39°C showed hatchability of 74.14%, which is about 5% lower than the hatchability of eggs kept at 30°C. The temperature (39°C) used in this experiment is, therefore, probably not in the optimum range for Tribolium development. The radiosensitivity of horizontal rotated eggs ($R_{\frac{1}{2}} = 4$ rpm) appears to be increased. Statistical analysis of the difference in hatchability between irradiated horizontal stationary and irradiated horizontal rotated eggs gives a "t" value of 2.96 and a probability of 0.01, indicating the difference is significant.

D. Growth and development through different life stages under gravity compensation.

As a whole, there is no growth within an egg, and growth during insect development is restricted to larval development. During this larval feeding period, there is a deposition of all the mass necessary for the final adult. The larval stage, in general, can be considered an extension of the embryonic stage. As larvae usually show a more definite sensitivity to gravity and have a larger body size than eggs, some physiological functions of larvae may be gravity-dependent. The growth and metamorphosis of insects are known to be controlled by some neurohormones, e.g. ecdyson and juvenile hormone, which are transferred to target tissues through the blood system. It seems possible that, in a weightless state, larvae might have difficulty maintaining the homeostatic state inside their bodies and might show abnormal development. To investigate this possibility, eggs and larvae of various ages, placed in a clean glass tube (I.D. 1 cm) filled with flour medium were kept in the clinostat at 30°C till they reached adult stage. The number of normal adults at the conclusion of each experiment was checked; results of eleven experiments are shown in Figure 15. It was found that Tribolium eggs and young larvae (< 15 days old at 30°C) developed into adults successfully under compensated gravity. Larvae older than 15 days and young pupae less than 1 day old at 30°C, however, showed an increase in developmental failure under gravity compensation. This decrease of normal development in old larvae and young pupae is quite large and may be due to the effect of gravity compensation. Other mechanical factors, nevertheless, cannot be ruled out completely, since the rotation of specimen holders may displace and injure the Tribolium or medium.

E. Late effect of gravity compensation on radiosensitivity of adults.

Although Tribolium eggs seem to grow and develop successfully into adult stage under compensated gravity, based on the morphological appearance of outer body structures, some injuries to the structure and function of inner tissues and organs could be produced in the adult stage. A preliminary experiment was done to examine the radiosensitivity of adults developed under gravity compensation. Tribolium adults developed from egg stage in the clinostat at 30°C were collected, and one group was X-irradiated with a dose of 6.6 K rads. The mortality of adults was checked up to about 50 days postirradiation, and more than 200 adults were used for each experimental condition. Results are shown in Figure 16. Significantly higher mortality was found in X-irradiated adults that developed under gravity compensation. No difference in mortality was observed between the irradiated, vertically rotated group ($R_{\frac{1}{2}}: 4 \text{ rpm}$) and that X-irradiated, and non-rotated ($S_{\frac{1}{2}}: 0 \text{ rpm}$). Also the survival of nonirradiated groups did not show any significant difference. Tribolium beetles developed under gravity compensation, therefore, appear to be more sensitive to radiation, and possibly to other stresses.

F. Reproduction of Tribolium with gravity compensation.

As the reproductivity of higher organisms in the space environment is not well understood, some preliminary experiments have been done to study the effect of gravity compensation on egg production in Tribolium. Packed with 1 ml flour medium, four virgin female adults and one male adult were put in a clean glass tube (I.D. 1 cm), at each end of which a piece of sterile cotton was inserted in order to keep medium and beetles in place. For each experimental condition, duplicates were kept in the clinostat at 25°C for one week. The number produced and hatchability of eggs laid during this period were checked. After beetles were removed from the clinostat, they were transferred with medium into a clean glass vial and kept in a 25°C

incubator. Eggs were collected from these adults each day for 4 days, and their hatchability was checked. Results of a typical experiment are shown in Figure 17. Eggs collected in the first period were produced by beetles in the clinostat; each point in the graph represents eggs laid by 4 females. In general, both hatchability and the number of eggs produced by adults under compensated gravity ($R_{\perp \frac{1}{g}}$: 32 rpm and $R_{\perp \frac{1}{g}}$: 128 rpm) are comparable with that of vertically rotated ($R_{\parallel \frac{1}{g}}$: 32 rpm) and horizontal stationary ($S_{\perp \frac{1}{g}}$: 0 rpm) groups. The fluctuation of points in the graph is most likely due to the small size of samples used in this experiment. The fecundity and fertility of beetles, therefore, appear not to be affected by gravity compensation.

VI. Discussion

The basic goal of this clinostat work is to reproduce the observed results of the Biosatellite-II experiment at ground level: to systematically determine the synergistic effects on biological development of weightlessness of higher organisms and radiosensitivity, and to study the mechanisms that may be involved in the observed synergistic effect. Although extensive studies on the effect of gravity compensation and radiosensitivity on Tribolium eggs and pupae have been made, at present it is evident that our experimental results have not answered the question raised by the Biosatellite-II experiment, i.e., what causes the observed synergistic effect? On the contrary, more questions have been raised.

Experimental results from more than 25,000 eggs suggest that the embryonic development of Tribolium eggs is not sensitive to gravity compensation. It is unlikely that this conclusion is a result either of the eggs being past the stage sensitive to gravity compensation or of not being exposed long enough to a high degree of compensated gravity. For most experiments, eggs were less than 8 hours old (24°C) at the time they were placed in the clinostat. The number of cells in the egg could not have been more than 256 or 2^8 with the egg still at early cleavage stage. By the time eggs were removed from the clinostat, larvae were formed in the egg, since, in all experiments no significant hatching delay was observed when compared with the incubated controls. Almost the entire embryonic development was, therefore, subjected to gravity compensation. The fact that experiments at various rotation speeds from 8 to 128 rpm gave identical results, further supports the finding that cellular growth, differentiation, and morphogenesis in the egg can proceed almost normally under conditions of gravity compensation.

The possibility was investigated that compensated gravity might modify the radiosensitivity of Tribolium eggs. Our experimental results showed no change in radiosensitivity when eggs were X-irradiated either before exposure to compensated gravity or during conditions of gravity compensation. This result suggests that gravity compensation alone may not induce any significant potential injury to cells. However, gravity compensation cannot be totally neglected as a possible physical stress since under extreme adverse conditions, it might enhance the biological effects of other environmental factors. At 39°C, a temperature close to lethal for Tribolium, eggs irradiated under gravity compensation showed significant decrease in hatchability compared with those irradiated at 1 g.

The insensitivity of Tribolium eggs to gravity compensation could be genetically controlled, since there is no evidence that eggs laid by adults show any specific orientation with respect to gravitational force. The repair mechanism also could be involved. The high rate of cell proliferation during embryonic development could efficiently repair injuries induced by gravity compensation. Because the radiosensitivity of eggs changes so fast with aging, it has been difficult to study the repair kinetics of eggs. Furthermore, the mechanism involved in minimizing the effect of gravity compensation in eggs is still unknown.

Since a synergistic effect of gravity compensation was observed in pupae, it is natural to assume gravity compensation affects the more developed organism, i.e., pupae. Many experiments, therefore, were done with young pupae, despite all the negative findings in eggs. Without radiation, 1-day old (30°C) pupae appeared to develop quite normally under gravity compensation, and no increase of deformity was found in adults. This result seems to be in agreement with the Biosatellite-II experiment. However, when pupae were gamma-irradiated under conditions

of compensated gravity, no synergistic effect was observed. An enhancement of radiosensitivity in pupae under compensated gravity was, nevertheless, obtained when the clinostat temperature was set at 39°C. Again, it indicates that, although compensated gravity is a weak stress to Tribolium, it can increase the severity of injuries caused by other physical stresses under certain conditions. A summary of these results and effects observed in space flight is given in Table 7.

Results obtained from pupae experiments using the clinostat thus strongly suggest that, in order to explain the synergistic effect found in the Biosatellite-II experiment, some additional factors besides weightlessness should be taken into consideration. Since the relatively high flux of cosmic rays is a unique environmental factor in space, and since no measurement of the flux of cosmic rays was made for the Tribolium experiment during the Biosatellite-II flight, it is possible that the observed synergistic effect may have been due to the radiation of heavy particles. We have studied the relative biological effectiveness (RBE) of heavy ions at the HILAC, and some of the experimental results are shown in Figure 18, 19, and 20. It can be seen that the survival curve changes from sigmoidal to exponential as the atomic number of particles increases from 4 to 12. Therefore, a single hit of high-LET particle, e.g. C^{12} , can produce a lethal effect in eggs. The RBE and HZE particles, as determined from these data, can be as high as 1,000 for a single particle. The high effectiveness of HZE particles in inducing lethal damage in organisms makes this proposed explanation for the Biosatellite-II experimental results attractive.

It should be noted, however, that other space factors could also have caused the synergistic effect observed in the Biosatellite-II experiment. We found earlier, for instance, that Tribolium pupae adequately packaged were not sensitive to normal sound and vibration but were very sensitive to

ultrasound. The ground control experiment conducted with vibration after the flight had the apparent vibration spectrum which existed on Biosatellite-11, except that ultrasonic frequencies, in our opinion, were not adequately controlled. Ultrasonic waves produced during the flight might thus have enhanced the radiosensitivity of Tribolium pupae. The possibility that weightlessness may contribute some of the observed increase of abnormalities in pupae irradiated in space, however, cannot be completely neglected: there is evidence indicating that gravity compensation and weightlessness are similar but not the same in their effects (10,11). How much enhancement of radiosensitivity is due to weightlessness, or heavy ions of cosmic rays, or ultrasonic waves can be answered only by conducting additional well-designed experiments in space. If the NASA space program will include some long-term orbital and interplanetary flights in the near future, we believe it is important to have this question answered, so that the potential risk to astronauts can more accurately be estimated.

The development of Tribolium under compensated gravity through egg, larval, and pupal stages was also studied, and our results showed that eggs can develop successfully into adults under gravity compensation. The finding that aged larvae (> 15 days old at 30°C) and young pupae (less than 1 day old at 30°C), when exposed to gravity compensation, did not develop into normal adults appears to be anomalous. It will certainly help clarify these results if some similar experiments can be carried out in space.

The fecundity and fertility of Tribolium beetles also showed no significant change when adults were subjected to gravity compensation. However, our data are preliminary, and beetles were kept under compensated gravity for only a relatively short time. It should not be assumed that the reproductivity of Tribolium will not be altered when this organism is subjected to weightlessness for a longer period. A

better specimen holder recently has been designed and constructed in our laboratory in order to study the dynamics of population under gravity compensation (see Figure 21). It is hoped that this type of experiment will be conducted not only with the clinostat but also in space.

The increase in radiosensitivity of beetles developed under gravity compensation from egg stage is of interest. Since results are preliminary, more experiments need to be done in order to confirm this finding and to understand the mechanisms involved. Should this increase in radiosensitivity be confirmed, the potential harmful effect of weightlessness to organisms on long-term space flight must be considered more severe than expected.

VII. A Suggestion For Future Studies

Through the effort of many years' space exploration, our understanding about space has reached an unprecedented level. The ability of humans to travel through outer space has been well demonstrated by the success of Apollo flights to the moon. All the space trips having taken place so far, however, are relatively short-term compared with a flight to Mars or other planets in the solar system. A visit to other planets in and out of our solar system will certainly occur sometime in the future, for man is adventurous and curious by nature. Without continually satisfying our curiosity about the unknown, our civilization cannot evolve to a higher level; our life will be static and less meaningful. Exploration of the universe is therefore inevitable and may become a significant part of human destiny.

Some early Biosatellite experiments have shown that the space environment may not be completely harmless to living organisms. For a long-term space flight, the question of how much potential damage or risk astronauts will take must be answered in order to ensure the success of each mission. Unfortunately, our understanding about the potential effect of each space environmental factor is very limited, partly because it is impossible to isolate each factor in space. Using the facilities of the clinostat and the BEVALAC, it is now possible to study two important space factors, i.e., weightlessness and cosmic rays, at ground level. The research work conducted by our group in the past several years has been designed to gain more complete knowledge of the responses of organisms to gravity compensation and HZE particles.

Although an extensive investigation has been made on the immediate effects of gravity compensation combined with radiosensitivity on egg and pupal development, our data on long-term effects are preliminary due to the limitation of time and manpower; further studies should be pursued.

Completion of the BEVALAC at Berkeley has extended the energy range of heavy ions which now can be studied at ground level. It is hoped that particles as heavy as iron will be accelerated within the next few years. A continuation of the present HZE-particle research program will give us some basic information on the biological effects of cosmic rays, and an understanding of their potential hazards can be very useful in finding means for radiation protection in the future.

Since many physical and chemical factors are present in a spaceship, a study on the combined effect of several environmental stresses is important and necessary for an accurate understanding of potential hazards in space. Our results have shown that the combined effect of stresses can often be greater than the sum of effects of each factor (6). More experiments like this should be done in order to complete our knowledge in this field.

Most experiments at ground level are conducted essentially for the purpose of improving our understanding of experimental results obtained from previous space flights, and some studies are aimed at examining various aspects of biological response in the space environment. The clinostat cannot provide true weightless conditions and cannot be used to study responses in large animals, e.g. rats and dogs. Many other space factors cannot be exactly duplicated at ground level at present, space-flight experiments are therefore necessary in order to confirm the validity of our experimental findings in the clinostat work. Space-flight experiments and research studies with the clinostat and heavy-ion accelerators are complementary to such space flights.

The space shuttle program will be ideal for conducting in space some of the complicated biological experiments done with the clinostat and certainly will rapidly enhance our knowledge of space biology. The abundant information available on Tribolium makes it a good candidate for space-

flight experiments. Adult beetles have a relatively long life span (1 to 2 years at 30°C); their radiosensitivity changes slowly with age; and they require a simple environment in which to live; these considerations suggest Tribolium for biodosimetry use. Injuries induced by space environmental stresses to man are often nonlethal and cannot be assessed within a short time. Furthermore, there is no suitable physical device that can detect the interaction of very high-energy cosmic rays with living cells and tissues. Some of the unexplained results of space-flight experiments have been attributed to super energetic cosmic rays by Akoev and Yurov (1974)(12). By checking the life span, the radiosensitivity, and other physiological functions of adults used for biodosimetry, the residual injury produced by stresses in space can then be measured. This information can be useful in determining any injuries to astronauts following a space flight.

VIII. SUMMARY

For investigating the effects of gravity compensation and radiation on developing Tribolium, a pair of "1- π " clinostats, one equipped with a Cs¹³⁷ gamma-ray source, were designed and built in our laboratory. These clinostats can provide a gravity compensation of $10^{-3}g$ to $10^{-4}g$. Rotation speeds of the clinostats can be varied from 2 rpm to 128 rpm, and air temperature and humidity are controlled.

Our experimental results showed that embryonic development of Tribolium eggs and the morphogenesis of pupal wings were not affected by gravity compensation at temperatures from 24° to 34°C. Radiation given prior to gravity compensation, also caused no synergistic effect on the embryonic development of Tribolium eggs. These findings are in agreement with our earlier results from the Biosatellite-II experiment with Tribolium.

When pupae were irradiated under compensated gravity at 25°C, no synergistic effect on wing malformation was observed, except at the highest dose (3650 rads). However, pupae irradiated at high temperature, (38°-39°C), under gravity compensation, did show a significant synergistic effect. An increase of radiosensitivity was also found in pupae under gravity compensation when they were subjected to low temperature (3°C) before receiving gamma-irradiation.

Tribolium eggs and young larvae ($\leq$ 15 days old at 30°C) successfully developed into adults under compensated gravity. Beetles developed under gravity compensation from egg stage, however, showed higher radiosensitivity than controls, suggesting that some potential injuries to the developing organism can be induced by gravity compensation.

Fecundity and fertility of beetles appeared not to be affected by gravity compensation.

Since these experiments were designed to study the short-term effects of gravity compensation on the development of Tribolium, an organism exposed to

weightlessness for a long period might show quite different results. The abundance of information available on Tribolium genetics and radio-sensitivity and on Tribolium response to weightlessness, temperature, and magnetic field, makes it a suitable organism for studying the effects of long-term space flight on living organisms.

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Table 1. Development of T. confusum Pupae (24-hr. old at 30°C) under Compensated Gravity

<u>Expt. no.</u>	<u>Temperature in clinostat</u>	<u>Condition</u>	<u>No. pupae</u>	<u>% Normal adults</u>
129	34°C	Horizontal stationary (S ₁ G: 0 rpm)	101	93.69
		Horizontal rotated (R ₁ G: 4 rpm)	150	98.04
131	34°C	Horizontal stationary (S ₁ G: 0 rpm)	100	100
		Horizontal rotated (R ₁ G: 4 rpm)	150	99.50
132	34°C	Horizontal stationary (S ₁ G: 0 rpm)	198	93.90
		Horizontal rotated (R ₁ G: 4 rpm)	172	93.84

Table 2. Effect of Radiation and Gravity Compensation on Wing Development

<u>Temperature in Clinostat</u>	<u>Dose Rate of ^{137}Cs γ-rays</u>	<u>Dose (rads)</u>	<u>Condition</u>	<u>No. Pupae</u>	<u>% Normal Adults</u>	<u>Standard Deviation (σ)</u>	<u>Probability (P)</u>
25 $\pm$ 1° C	10 R/min.	0	Horizontal stationary (S $\uparrow$ g: 0 rpm)	190	96.84	1.27	-
		2600	Horizontal stationary (S $\uparrow$ g: 0 rpm)	408	84.80	1.78	} 0.1 $\leq$ P $\leq$ 0.2
			Horizontal rotated (R $\uparrow$ g: 4 rpm)	609	88.18	1.31	
		3000	Horizontal stationary (S $\uparrow$ g: 0 rpm)	207	34.78	3.31	} 0.4 $\leq$ P $\leq$ 0.5
			Horizontal rotated (R $\uparrow$ g: 4 rpm)	201	38.31	3.43	
		3200	Horizontal stationary (S $\uparrow$ g: 0 rpm)	402	25.13	2.16	} 0.3 $\leq$ P $\leq$ 0.4
			Horizontal rotated (R $\uparrow$ g: 4 rpm)	602	22.76	1.71	
		3650	Horizontal stationary (S $\uparrow$ g: 0 rpm)	198	7.07	1.82	} 0.001 $\leq$ P $\leq$ 0.01
			Horizontal rotated (R $\uparrow$ g: 4 rpm)	191	1.05	0.74	

Table 3. Effect of Radiation, Temperature and Compensated Gravity on the Development of *T. confusum* Pupae (24-hr old at 30° C)

<u>Temperature in Clinostat</u>	<u>Dose Rate of ^{137}Cs γ-rays</u>	<u>Dose (rads)</u>	<u>Condition</u>	<u>No. pupae</u>	<u>% Normal Adults</u>	<u>Standard Deviation(σ)</u>	<u>Probability(P)</u>
39° C	2 R/min.	0	Horizontal stationary (S_{1g} : 0 rpm)	38	100	-	
		2194	Horizontal stationary (S_{1g} : 0 rpm)	403	53.10	2.49	} 0.01 < P < 0.02
			Horizontal rotated (R_{1g} : 4 rpm)	406	44.34	2.47	
		2628	Horizontal stationary (S_{1g} : 0 rpm)	405	52.10	2.48	} 0.1 < P < 0.2
			Horizontal rotated (R_{1g} : 4 rpm)	504	47.02	2.22	
		2947	Horizontal stationary (S_{1g} : 0 rpm)	403	38.46	2.42	} 0.02 < P < 0.05
			Horizontal rotated (R_{1g} : 4 rpm)	604	31.46	1.89	
		3100	Horizontal stationary (S_{1g} : 0 rpm)	198	39.90	3.48	} 0.1 < P < 0.2
			Horizontal rotated (R_{1g} : 4 rpm)	202	33.66	3.33	
		3456	Horizontal stationary (S_{1g} : 0 rpm)	399	35.34	2.39	} 0.4 < P < 0.5
			Horizontal rotated (R_{1g} : 4 rpm)	800	33.13	1.66	

Table 4. Effect of Radiation, Temperature and Rotation on Wing Abnormalities of T. confusum.

Temperature in <u>Clinostat</u>	Dose <u>Rate</u>	Dose (<u>rads</u>)	<u>Condition</u>	<u>No. pupae</u>	% Normal <u>Adults</u>	Standard <u>Deviation</u> (σ)	<u>Probability</u> (P)
38°C	10 R/min	0	Horizontal stationary ($\vec{S_{1E}}$: 0 rpm)	245	95.10	1.26	} 0.05 < P < 0.1
			Horizontal rotated ($\vec{R_{1E}}$: 4 rpm)	228	91.22	1.87	
		1700	Horizontal stationary ($\vec{S_{1E}}$: 0 rpm)	106	91.51	2.71	} 0.8 < P < 0.9
			Horizontal rotated ($\vec{R_{1E}}$: 4 rpm)	106	92.45	2.57	
		2000	Horizontal stationary ($\vec{S_{1E}}$: 0 rpm)	312	73.08	2.51	} 0.1 < P < 0.2
			Horizontal rotated ($\vec{R_{1E}}$: 4 rpm)	313	67.41	2.65	
		2200	Horizontal stationary ($\vec{S_{1E}}$: 0 rpm)	205	66.83	3.29	} 0.05 < P < 0.1
			Horizontal rotated ($\vec{R_{1E}}$: 4 rpm)	206	57.77	3.44	
		2400	Horizontal stationary ($\vec{S_{1E}}$: 4 rpm)	310	58.07	2.80	} 0.1 < P < 0.2
			Horizontal rotated ($\vec{R_{1E}}$: 4 rpm)	311	51.77	2.83	

Table 4 cont'd.

<u>Temperature in Clinostat</u>	<u>Dose Rate</u>	<u>Dose (rads)</u>	<u>Condition</u>	<u>No. pupae</u>	<u>% Normal Adults</u>	<u>Standard Deviation(σ)</u>	<u>Probability(P)</u>
		2700	Horizontal stationary ($S_{\perp} \vec{g}$: 0 rpm)	315	39.05	2.75	} $P < 0.001$
			Horizontal rotated ($R_{\perp} \vec{g}$: 4 rpm)	315	23.49	2.39	
		0	Vertical stationary ($S_{\parallel} \vec{g}$: 0 rpm)	204	96.57	1.27	} $0.3 < P < 0.4$
			Vertical rotated ($R_{\parallel} \vec{g}$: 4 rpm)	132	94.70	1.95	
		2400	Vertical stationary ($S_{\parallel} \vec{g}$: 0 rpm)	204	53.43	3.49	} $0.3 < P < 0.4$
			Vertical rotated ($R_{\parallel} \vec{g}$: 4 rpm)	199	57.29	3.51	
		2410	Vertical stationary ($S_{\parallel} \vec{g}$: 0 rpm)	202	54.95	3.50	} $0.9 < P$
			Vertical rotated ($R_{\parallel} \vec{g}$: 4 rpm)	204	55.39	3.48	

Table 5. Embryonic Development of *T. confusum* Eggs (8-hr. old at 24° C)
Under Compensated Gravity at 34° C

<u>Expt. No.</u>	<u>Condition</u>	<u>No. Eggs</u>	<u>No. Larvae</u>	<u>% Hatchability</u>
51	Horizontal stationary	808	667	82.55
	(S ₁ G: 0 rpm)			
	Horizontal rotated	806	680	84.37
	(R ₁ G: 32 rpm)			
54	Horizontal stationary	694	553	79.68
	(S ₁ G: 0 rpm)			
	Horizontal rotated	816	651	79.78
	(R ₁ G: 32 rpm)			
62	Horizontal stationary	600	510	85.00
	(S ₁ G: 0 rpm)			
	Horizontal rotated	600	513	85.50
	(R ₁ G: 32 rpm)			

Table 6. Effect of Cyclic Irradiation (15 R/min) and Gravity Compensation (R_{1g}^+ : 4 rpm)
On Hatchability of T. confusum Eggs (8-hr. old at 24° C)

<u>Temperature in Clinostat</u>	<u>Dose (R) of γ-rays</u>	<u>Condition</u>	<u>No. eggs used</u>	<u>% Hatchability</u>	<u>Standard Deviation (σ)</u>
39±1°C	0	Horizontal stationary + (S_{1g} : 0 rpm)	116	74.14	4.07
	5.	Horizontal stationary + (S_{1g} : 0 rpm)	400	33.00	2.35
	553	Horizontal rotated + (R_{1g} : 4 rpm)	800	24.88	1.53

Table 7. Summary of Biosatellite II, vibration, and radiation clinostat experimental results.

Name of Expt.	Condition	Temp. (F°)	Pre-Irrad. Yes	Dose rate (R/hr)	Irrad. during weightless Yes	Dose rate (R/hr)	Total Dose (R)	Sample size	Wing abnormalities (%)	Standard deviation (σ)	Probability (P)
Biosat. II	Earth control	84	Yes	6750	Yes	22.54	2319	240	44.8	3.2	0.025
	Flight	84	No	0	Yes	17.56	755	120	2.6	1.4	} not signif
	E. control	86	No	0	Yes	23.95	1030	120	1.7	1.2	
	Flight	84	Yes	6750	No	<3	1350	240	19.0	2.5	} not signif
	E. control	85	Yes	6750	No	<3	1350	240	16.8	2.4	
	Flight	84	No	0	No	<3	<3	120	4.3	1.8	} not signif
	E. control	85	No	0	No	<3	<3	120	3.7	1.7	
Vibration acceleration test	Test	85	Yes	6750	Yes	22.98	2338	126	42.4	4.4	} not signif
	control	85	Yes	6750	Yes	23.56	2364	123	53.3	4.5	
	Test	85	No	0	Yes	22.09	950	60	1.7	1.7	} not signif
	control	85	No	0	Yes	22.32	960	57	5.5	3.0	
	Test	85	Yes	6750	No	<3	1350	123	43.2	4.5	} not signif
	control	85	Yes	6750	No	<3	1350	118	39.4	4.5	
	Test	85	No	0	No	<3	<3	63	9.5	3.7	} not signif
	control	85	No	0	No	<3	<3	62	0.0	0	
Clinostat	Horizontal stationary	77	No	0	No	0	0	190	3.2	1.2	} 0.1 ≤ p ≤ 0.2
	H. stat.	77	No	0	Yes	600	2600	408	15.2	1.7	
	H. rotated	77	No	0	Yes	600	2600	609	11.8	1.3	
	H. stat.	77	No	0	Yes	600	3000	207	65.2	3.3	
	H. rotated	77	No	0	Yes	600	3000	201	61.6	3.4	} 0.4 ≤ p ≤ 0.5
	H. stat.	77	No	0	Yes	600	3200	402	74.8	2.1	
	H. rotated	77	No	0	Yes	600	3200	602	77.2	1.7	} 0.3 ≤ p ≤ 0.4
	H. stat.	77	No	0	Yes	600	3650	198	92.9	1.8	
	H. rotated	77	No	0	Yes	600	3650	191	98.9	0.7	} .001 ≤ p ≤ 0.01

Table 7. Summary of Biosatellite II, vibration, and radiation clinostat experimental results (cont.).

Name of Expt.	Condition	Temp. (F°)	Pre-Irrad.	Dose rate (R/hr)	Irrad. during weightless	Dose rate (R/hr)	Total Dose (R)	Sample size	Wing abnormalities (%)	Standard deviation (σ)	Probability (P)
Clinostat	H. stat.	100	No	0	No	0	0	245	4.9	1.2	} 0.05 ≤ p ≤ 0.1
	H. rotat.	100	No	0	No	0	0	228	8.7	1.8	
	H. stat.	100	No	0	Yes	600	1700	106	3.4	2.7	} 0.8 ≤ p ≤ 0.9
	H. rotat.	100	No	0	Yes	600	1700	106	7.6	2.5	
	H. stat.	100	No	0	Yes	600	2000	312	26.9	2.5	} 0.1 ≤ p ≤ 0.2
	H. rotat.	100	No	0	Yes	600	2000	313	32.6	2.6	
	H. stat.	100	No	0	Yes	600	2200	205	33.1	3.2	} 0.05 ≤ p ≤ 0.1
	H. rotat.	100	No	0	Yes	600	2200	206	42.3	3.4	
	H. stat.	100	No	0	Yes	600	2400	310	41.9	2.8	} 0.1 ≤ p ≤ 0.2
	H. rotat.	100	No	0	Yes	600	2400	311	48.2	2.8	
	H. stat.	100	No	0	Yes	600	2700	315	60.9	2.7	} p ≤ 0.001
	H. rotat.	100	No	0	Yes	600	2700	315	76.5	2.3	
	Vertical stationary	100	No	0	No	0	0	204	3.4	1.2	} 0.3 ≤ p ≤ 0.4
	Vertical rotated	100	No	0	No	0	0	132	5.3	1.9	
	Vertical stationary	100	No	0	Yes	600	2410	202	45.0	3.5	} 0.9 ≤ p
	Vertical rotated	100	No	0	Yes	600	2410	204	44.6	2.4	

FIGURE CAPTIONS

- Figure 1. Wing abnormalities of flour beetles irradiated at pupal stage.
- Figure 2. Sketch of floor plan for the clinostat-irradiator housing complex.
- Figure 3. The air conditioner, temperature and humidity recorders, and rotation-rate control panels are shown in this picture.
- Figure 4. Front view of clinostat contained within an environmental chamber.
- Figure 5. Front view of irradiator; the clinostat is irradiated in horizontal position.
- Figure 6. A view of clinostat and irradiator for vertical irradiation.
- Figure 7. A complete holder system for pupae studies with clinostat.
- Figure 8. Effect of low temperature and gravity compensation on the wing development of gamma-irradiated pupae.
- Figure 9. Effect of low temperature on the radiosensitivity of Tri-bolium confusum pupae.
- Figure 10. Life cycle of Tribolium confusum.
- Figure 11. A holder system for Tribolium confusum egg studies with clinostat.
- Figure 12. Effect of various rotation rates on embryonic development of X-irradiated Tribolium confusum eggs (8 hours old at 30°C).
- Figure 13. Effect of gravity compensation and gamma-irradiation on the hatchability of Tribolium confusum eggs. Standard deviation is less than 2%.
- Figure 14. Response of Tribolium confusum eggs of various ages to gravity compensation and gamma-irradiation. Standard deviation is less than 2%.
- Figure 15. Development of Tribolium confusum under gravity compensation through various life stages.

Figure 16. Survival of X-irradiated Tribolium confusum beetles developed under conditions of gravity compensation from egg to adult stage.

Figure 17. Effect of gravity compensation on fecundity and fertility in Tribolium confusum adults.

Figure 18. Hatchability of Tribolium confusum eggs (6 hours old at 30°C) irradiated with various low-energy heavy ions.

Figure 19. Ninety percent lethal dose (fluence) of heavy ions of various energy and atomic number.

Figure 20. Relative biological effectiveness of heavy particles of various energy and atomic number.

Figure 21. A clinostat holder designed for studying the dynamics of Tribolium population under gravity compensation.

XBC 670-5718

Figure 1

FLOOR PLAN OF CLINOSTAT-IRRADIATOR HOUSING COMPLEX

- A, B, C, D, G : incubator or refrigerator units
 E : sink
 F : fume hood
 H : irradiator control console
 I : ovens
 J : power console for both clinostats
 K : temperature and relative humidity recorders
 L : Aminco-Aire conditioner
 M : analytic balance

XBL 756-1439

Figure 2

CBB 725-2764

Figure 3

CBB 716-2725

Figure 4

CBB 716-2727

Figure 5

CBB 716-2729

XBB 699-6005

Figure 7

XBL 754-851

Figure 8

XBL 754-852

Figure 9

CBB 735-3269A

Figure 10

XBB 7011-5356

Figure 11

EFFECT OF VARIOUS RPM ON 8 HR-OLD T. CONFUSUM EGGS

XBL 755-1374

Figure 12

XBL 756-1616

Figure 13

NBL 754-R4K

Figure 14

XBL 754-850

Figure 15

Figure 16

SUMMARY OF EGG PRODUCTION AND HATCHABILITY
FROM ADULTS ROTATED IN CLINOSTAT 7 DAYS ($40,10^6$ IN EACH GROUP)

XBL 754-847

Figure 17

HATCHABILITY OF T.CONFUSUM EGGS vs HEAVY ION and X-RAY IRRADIATION

XBL 755-1971

Figure 18

NBL 755-1372

Figure 19

XBL 755-1373

Figure 20

CLINOSTAT HOLDER FOR POPULATION STUDIES

XBL 749-1520

Figure 21