EFFECT OF LIGHT: DARK CYCLES ON LONGEVITY IN DROSOPHILA MELANOGASTER

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ABSTRACT

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IN DROSOPHILA MELANOGASTER

By

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In order to test the hypothesis that exogenous light cycles act as a chronometer in the aging process, Drosophila were subjected to one of three different day:night cycling conditions. One group (short cycle) received cycles of 11 hours of light, 11 hours of dark; the second group (normal cycle) received 12 hours of light, 12 hours of dark; and the third group (long cycle) 13 hours of light, 13 hours of dark.

No significant differences in longevity were found between groups. There was a significant difference between sexes ($p < .01$) with females living longer than males.

The importance of further research to determine the endogenous effect of the exogenous light cycles in order to understand the results is discussed. Also, implications of the results for aging theories are considered. If future work finds that altering cycles affects metabolic rate, the experimental results have important disconfirming significance for longevity theories which depend on a "burn out" notion.

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By

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A THESIS

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DEDICATION:

To my mother and father.

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INTRODUCTION

Virtually all behavioral, physiological, and other functions of organisms exhibit some type of rhythmicity. Many of these rhythms are circadian, i.e., about 24 hours, although lunar, annual, and other rhythms occur for some functions. Most researchers agree that circadian rhythms are "innate"; they occur spontaneously and maintain themselves without external stimulus cues. (Bunning (1967) has a good review of circadian rhythm research.) This naturally occurring rhythm, however, can be entrained, i.e., made to conform to some externally imposed standard, provided the standard does not deviate greatly from 24 hours. The most powerful entraining agent, or synchronizer, is light. By giving a periodic light flash in a normally dark room, or having light on for a certain amount of time, followed by lights off, etc., an experimenter can bring most rhythms under external control.

Recently a controversy has arisen regarding the role of rhythms in development. Most work has been done using Drosophila as the experimental animal. Development through various pre-adult stages is studied, with emergence of the adult from the pupae case (i.e., eclosion) as the end point.

The question is, does an externally imposed rhythm effect the organism's rate of development. In particular, can time to eclosion be shortened or lengthened? An obviously related question is: Does the light:dark cycle have an effect on the organism's longevity? The effect on longevity is the concern of this thesis. However, as the question had never been directly investigated, prior to this thesis, it is necessary to first examine the most relevant research concerned with similar problems, that is, research on (1) effect of light-dark cycles on pre-adult developmental rate and (2) factors affecting longevity.

In 1964, Pittendrich and Minnis published research suggesting that the light cycle did not affect developmental rate in Drosophila. They felt that the importance of the light cycle is simply that it acts as a cue as to when the fly should emerge. For example, the fly's development could actually reach a stage adequate for eclosion at any time during the scotophile (darkness) phase. The "behavioral act" of eclosion, however, is strongly inhibited by the scotophile phase of the endogenous cycle. As a result, at dawn (the beginning of the photophile phase) a large number of flies emerge giving what is known as an "eclosion peak."

Additional studies strengthened Pittenrigh and Minnis's position that light:dark cycles do not affect development, but merely signal when emergence can occur. Skopik and Pittendrigh (1967) used populations of Drosophila.

All populations were developmentally synchronous to the extent that all individuals were collected within a five-hour interval at the pre-pupae stage (before tanning of the cuticle). The circadian rhythms of the individual pupae of each population were synchronized by transferring them from the constant light (L:L) condition in which they were raised to a constant dark (D:D) condition. The transfer of each population was made at a different stage of its development. The different populations, although developmentally the same, emerged as much as 17 hours apart (median times).

The authors maintained that the transfer from light to dark synchronized the circadian oscillations of the individual pupae with each other, and that the synchronized oscillation continued without additional stimuli. Such a synchronized oscillation may be initiated at any time during pupal development. The important theoretical point is that subsequent emergence time is limited to a restricted phase in the circadian oscillation. In fact, emergence time (hours from onset of pupation to eclosion) can be predicted by the formula: $E = 15 \text{ hours} + nr$, where n is a number of periods and r is the length of the period, about 24 hours.

As an example of this formulation, suppose one culture was transferred to darkness at 12 hours after the onset of pupation. Fifteen hours after this transfer (thus at 12 + 15 = 27 hours of "pupal age") the pupae would begin a rhythmic "counting" of 24 hour periods. The beginning of

each period (the pupae's subjective dawn) would be a potential emergence time, assuming that the pupae were otherwise, developmentally, ready. Such developmental readiness occurs somewhere around 200 hours of pupal age. Thus, for this culture potential eclosion times would be 27 hours, 51, 75, 99, 147, 171, 195, 219, . . . hours. In Skopik and Pittendrigh's (1967) experiment eclosion was found to occur at 195.3 hours after pupation, when transfer to darkness was made 12 hours after pupation.

Similarly, a culture transferred to darkness at 102 hours of pupal age would begin its circadian rhythm at 117 hours (102 + 15) and continue: 141 hours, 165, 189, 213, 237, . . . hours. For this case eclosion occurred at 212.4 hours in the experimental situation. The rhythmicity occurs even though the cultures are kept in constant darkness after transfer from light. Thus, the rhythm is cued exogenously and then maintained endogenously.

Skopik and Pittendrigh's conclusion was "an oscillation is initiated at the LL/DD transition in all individuals irrespective of the developmental stage; subsequent emergence times are restricted to a limited phase of the oscillation so initiated" (p. 1864).

The other major researcher in this area came into conflict with Pittendrigh. Harker (1965) believed that pupal development was influenced by light-dark cycles and

that the crucial factor was the phase of the light cycle at which the organism entered a particular developmental phase.

Harker's (1965) experimental technique was to place pre-pupae on Petri dishes so the progress of each individual could be observed. From larval stage through pupal stage the flies were treated in one of four different ways:

1. 12:12, bright light:dim light until eclosion; observations *in situ*.
2. 12:12, bright light:dim light until last larval moult, then transferred to constant dark. Samples withdrawn every hour and not returned.
3. 12:12, light:dark until eclosion; observations *in situ* during light and by sample withdrawal method during dark.
4. 12:12, light:dark until last larval moult and then transferred to constant dark; observations by sample withdrawal methods.

Harker used 4 or 5 developmental "markers" (e.g., head eversion, yellow eye pigmentation) to follow developmental progress.

Treatments (1) and (3) above cause their subjects to experience more light:dark cycles than those in treatments (2) and (4). Emergence times were not affected by number of light:dark cycles. Harker's results led her to the conclusion that "there does not seem to be any correlation between the length of the developmental period and

the number of bright-dim cycles, or to any sequence of these cycles, but only to the light conditions during the first hour or two of the stage."

This conclusion was accepted by neither Pittendrigh (Skopik and Pittendrigh, 1967) nor Clayton (1968). What Harker had evidently overlooked is that an organism in L:D conditions subsequently switched to darkness continues to maintain its own internal circadian rhythm. Although the flies no longer have L:D, they continue to act, physiologically and otherwise, as if they did. Thus, the two groups (1) and (3) (more L:D cycles) are not different from (2) and (4) (less L:D cycles).

There may well be a correlation between number of light:dark (or dim) cycles and developmental rate but the evidence to date neither finds one nor presents convincing evidence that the variables are independent. Neither Pittendrigh nor Harker believe such a correlation exists. However, Pittendrigh's experiment was not designed to investigate whether number of light:dark cycles affected development. His research showed that Harker's work, failing to find a correlation, had a flaw that made it invalid.

Clayton (1968) approached the problem from a different angle. If the number of light:dark sequences might be important to development, a way must be found to vary the number of such cycles without repeating Harker's mistake. Clayton argued that there is some indirect evidence that

if an organism has many L:D cycles it will develop more rapidly than if it has fewer (he cited data from Shutze et al., 1962; and Minnis and Pittendrigh, 1968). To test this idea he reared Drosophila from egg to eclosion under different light:dark conditions: one group had long days (25 hrs., 9 mins.), one had short days (22 hrs., 52 mins.), one had constant light, and a fourth group had constant dark. The crucial idea is that the developmental process may be related not only to the passage of time per se but to the passage of day:night cycles. Thus, the expectation was that short cycle flies would emerge "earlier" (relative to a 24 hour day).

Clayton found earlier eclosion times for the short cycle group. He interpreted this result as evidence that the number of light:dark cycles effects developmental rate, e.g., the short cycle flies receive more cycles in a given time, therefore develop faster and emerge earlier.

Clayton also found that the constant light condition flies eclosed earlier than those raised under constant dark conditions. This lends some further support to his hypothesis. Aschoff's rule (Aschoff, 1960; Pittendrish, 1960) states that a diurnal animal in constant bright light conditions would maintain a faster endogenous circadian rhythm (i.e., greater frequency) than one in dim light or constant darkness. Therefore, in the same amount of time the constant light condition flies go through more cycles than the

constant dark condition ones. The early emergence of the constant light flies is consistent with the hypothesis that number of cycles affects developmental rate.

Clayton's conclusions have been challenged by Pittendrigh (1968) who felt the design did not sufficiently control for the "gating" phenomena. As explained earlier, Pittendrigh demonstrated that developmentally synchronous flies can emerge as much as 17 hours apart due to the fact that a population can only emerge during a restricted phase of its circadian oscillation. Pittendrigh believes the difference between the long and short cycle groups was due not to developmental differences, but to the long cycle group awaiting a later "gate" through which to emerge.

Clayton (1969) is now engaged in research to control for the gating phenomena. Presently, however, there is no unequivocal evidence which either proves or disproves the hypothesis that number of circadian cycles affects developmental rate.

The previous research, then, leads toward but does not attack the problem of interest here: does circadian period length (and thus number of cycles or "days") affect the longevity of Drosophila.

Just as research on circadian rhythms does not deal directly with this issue, neither does longevity research. However, a great deal of research has been done on longevity, particularly with Drosophila. One heavily investigated

condition is the effect of temperatures (e.g., Maynard Smith, 1958; Strehler, 1961; Hollingsworth, 1966; Bowler and Hollingsworth, 1966). Generally, the studies have tested hypotheses about how life-shortening in Drosophila is caused by high temperatures. A typical example of this type of research is the study by Strehler. He mentioned three possibilities: (1) at high temperatures there may be an increased rate of loss of thermosensitive structural and functional elements; (2) rate of utilization or destruction of some limiting non-replaceable metabolite is increased at higher temperatures; (3) there is an increased rate of accumulation of some deleterious factor. To test the first hypothesis he exposed D. melanogaster to extremely high temperatures for one hour. Those that survived showed a mortality rate similar to controls. He concluded:

> the results demonstrate that aging in Drosophila is not a result of high activation reactions, such as protein denaturation. Rather, the increase in mortality at high temperature must be a consequence of loss of functional units. . . . (p. 11).

Radiation effects on longevity have been another topic of investigation. For example, Lamb and Smith (1964) did research to investigate whether radiation reduces life span of insects primarily by causing mutations in the nuclei of somatic cells. This area of investigation is still controversial and no definite conclusions can be drawn as yet.

Sondhi (1965, 1966, 1967a, 1967b, 1968) has undertaken a series of studies of longevity which examined variables ranging from stress factors to temperature effects. In his stress experiments, for example, he removed hemolymph from Drosophila. Young flies received a replacement from an older donor or no replacement. In both cases there was a striking life shortening effect. When large amounts were removed, both life shortening and reduced fecundity occurred. He viewed the hemolymph removal as a stress factor which requires homeostatic adjustments on the part of the fly. The more severe the stress, the greater the effect on the organism's functioning.

The possibility that circadian cycles could have an effect on longevity has been given the barest recognition. Hollingsworth (1967), in describing the optimum (for increased longevity) environmental conditions under which he raised Drosophila, mentioned that they were given 24 hour day conditions. Thus, he indirectly recognized that circadian cycles could be of importance. On the other hand, Strughold (1965) suggested the possibility that one day man might be able to set his own day length (e.g., outside the solar system) and ignored any possible effects of non-twenty-four hour days.

If circadian cycle length is varied there are three reasonable outcomes. One, as suggested by Clayton (1968), is that development is speeded (relative to our 24 hour day)

when cycles are short and retarded when cycles are long. If the effect holds for the organism's total development, i.e., from birth to death, then those with short days would die earlier and those with long days later, than those with normal days. A second possibility is that when days are varied from 24 hours, there is a deleterious effect on the organism. Other research suggests such an outcome; for example, if rhythms are very deviant from 24 hours, it inhibits flowering in some plants (Went, 1962). If this applies to longevity, we would expect to find long and short cycle organisms dying earlier than those with normal cycles. A third reasonable possibility is, of course, that cycle length has no effect on longevity.

METHOD

Clayton (1968) examined the effect of non-twenty-four hour days on early development. Here the concern is with "adult development" or longevity, using the time span from eclosion to death, rather than oviposition or pupation to eclosion. Such an approach has two advantages: (1) The mean time span from eclosion to death (2 or 3 months) is much greater than that from oviposition or pupation to eclosion (a matter of days or hours). If there are effects of varied light:dark cycles, they might be more obvious when taken over the organism's whole life span. (2) Because of this, the "gating" problem will be avoided; that is, differences, if they are to be significant, must be greater than 24 hours.

Experimental Design

As shown in Table 1 the independent variables in the experiment were day length, sex, and housing (bottle vs. vial) condition. The design was primarily used to test effects of light:dark cycles of three different lengths on longevity of male and female *Drosophila*. Differences in longevity between vial and bottle housed flies as well as

Table 1. Experimental design; each cell shows number of Ss for that cell

	Day length for vials			Day length for bottles		
	26 hrs.	24 hrs.	22 hrs.	26 hrs.	24 hrs.	22 hrs.
Male	100	100	100	100	100	100
Female	100	100	100	100	100	100

interactions between the three independent variables were obtained; however these were incidental to the main purpose.

Subjects

The experimental subjects were Drosophila melanogaster, Oregon strain. There were a total of 1200 flies, 600 males and 600 females. Six hundred flies (300 males, and 300 females) were housed in glass vials 7 cm. long. Each vial contained about 4 cc. of agar media and held 5 flies of the same sex. Another 600 flies were housed in twelve half-pint milk bottles containing 10 cc of media.

The experiment was begun with all 1200 flies housed in vials. After two weeks it was decided to transfer half the flies (600) to the half-pint bottles. Reasons for the decision to make the transfer are explained in the procedure section. There were 50 flies in each of the twelve bottles, less those that had died since the beginning of the experiment. The exact number of flies in each bottle is shown in Table 2.

Table 2. Number of flies in bottles at transfer (in total there were 12 bottles, 2 assigned to each sex by condition groups)

Group	Flies per first bottle	Flies per second bottle	Total
Long/male	43	44	87
Short/male	38	39	77
Normal/male	42	43	85
Long/female	48	48	96
Short/female	46	47	93
Normal/female	47	47	94
Total			532

The transfer procedure was systematic, i.e., every other vial of flies was transferred to the bottle type housing. And, of course, conditions remained the same, that is, sex homogeneity of bottle mates and day length treatment were maintained for any given fly.

Apparatus

Three boxes of a type constructed by Clayton and described in greater detail elsewhere (Clayton, 1968) were used to hold the vials and the half-pint bottles (see Figure 1). Each box was covered with a rotatable shutter. When the shutter was verticle (open position) light entered the box. When the shutter was horizontal no light entered.

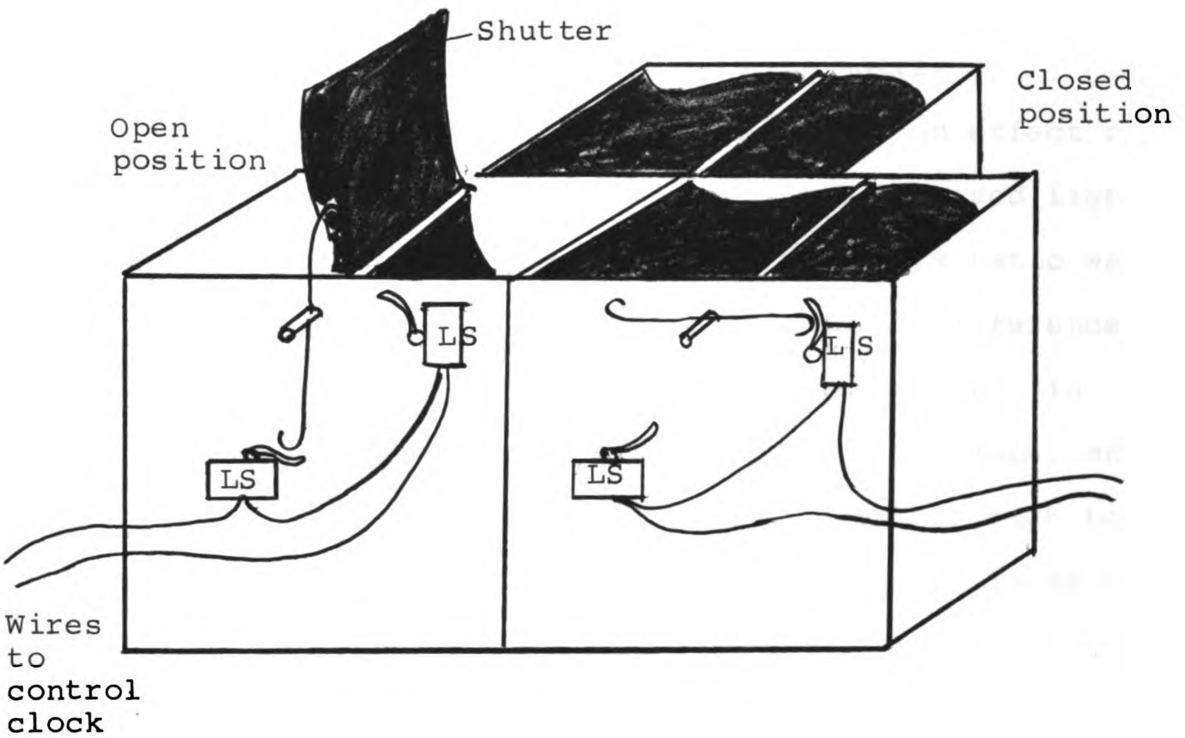

Figure 1. Motor driven Light:Dark boxes. At a time set on the control clock the shutter turns 90° simulating either dawn or dusk. Limiting switches (LS) check the travel of the shutter. Each box is controlled by a separate clock which automatically resets itself.

Synchronous motors, attached to control clocks, controlled each shutter, allowing a 15 minute "dawn" or "dusk" in light:dark transitions. At time zero the shutter would open (dawn); after the appropriate amount of time (11, 12, or 13 hours) the shutter would close (dusk) and remain closed for the dark period (11, 12, or 13 hours).

The amount of light energy could have an effect on development. Although each treatment group received light for different periods of time, the light to dark ratio was the same, i.e., 50%:50%. A possible source of difference, however, was the amount of light entering each box. In order to be reasonably sure the amounts were the same, an oscilloscope was used to measure light energy for each box. The detecting device was placed in a box and the clocks were set so that the shutter would go from open to close to open. (In the actual experiment, of course, once a shutter closed, dusk, it remained closed for the appropriate time period.) Figure 2 gives the change in light over time as the shutter closed and reopened. Inspection shows that the boxes were very close in amount of light received.

The boxes were placed in an upright refrigerator which maintained temperature at 25^{o} C $\pm$ 0.5^{o} C. The refrigerator contained two 25 watt fluorescent tubes which remained on constantly. The light:dark cycles were affected by the opening and closing of the shutters on the light:dark boxes.

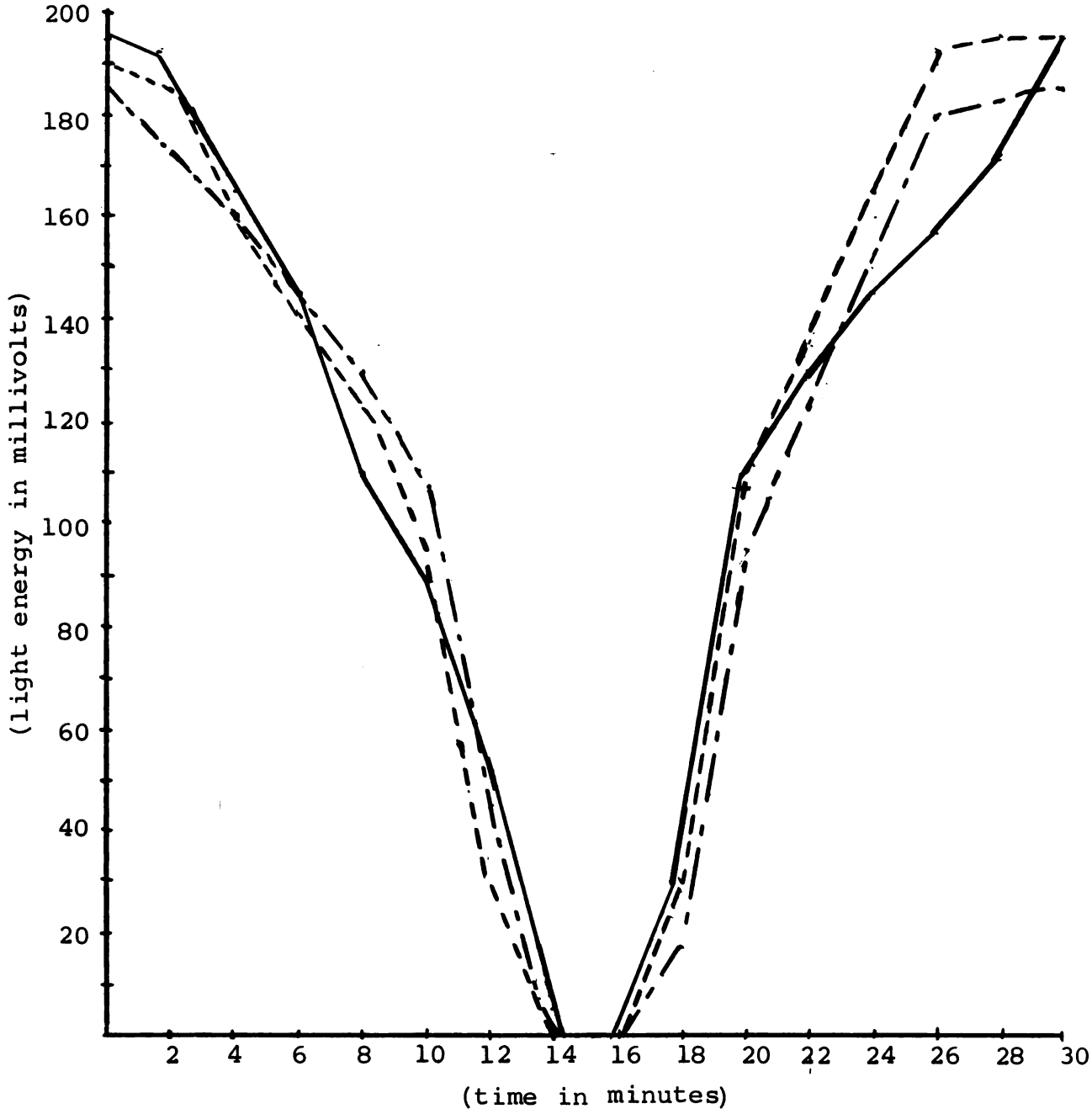

Figure 2. Light:Dark transition curves.

Procedure

Stock for the experiment was raised in such a way as to insure equality of age as far as possible.

Adult flies (of both sexes) were placed in half-pint jars containing agar between 9:20 and 10:00 a.m. for the purposes of egg laying. Adults were systematically removed (i.e., those placed in jar 1 were the first removed, those in jar 2 were the second removed, etc.) between 12:15 and 12:45 p.m. Thus, all eggs were laid between 9:20 a.m. and 12:45 p.m.

The eggs were incubated at 25^{o} C under constant light. Constant light was used in order to get a normal distribution of emergence time rather than the eclosion peak at dawn associated with day:night cycling. Some flies emerging during such a peak may be more developmentally advanced than others due to the gating phenomena discussed in the introduction (p. 8).

At 8:00 a.m. on the tenth day after laying, newly emerged adult flies were cleared from the bottles. Flies which emerged between 8:00 a.m. and 1:00 p.m. were collected for the experiment. There was an additional collection period of flies eclosing between 1:00 and 5:00 p.m. as a safety measure to insure that there would be enough flies for the experiment. In order to meet the subject number quota, 18 males from the later group were used. Six of these "late" males were used in each of the male treatment

groups (long, normal, short cycle lengths); the remaining 194 males of each male treatment group were from the first eclosion period. All females (200 per female treatment group) were from the first eclosion period. Thus, the experimental stock was the same age in terms of (1) being layed during a common three hour period, and (2) having emerged during a common five hour period (except for the 18 late males).

As the experimental flies were collected they were etherized, sorted according to sex, and placed in empty vials to recover. Recovered flies were then placed five to a vial (which contained media), and vials were stoppered with plastic foam plugs. All vials were placed in the light:dark boxes which were set on open (light condition). At 9:00 a.m. the following day, all boxes closed for the beginning of the first dark period.

The timing devices were set so that each box had its own light:dark cycle. One group received short cycles (11L:11D); one received normal cycles (12L:12D); and one received long cycles (13L:13D). These particular cycle lengths were chosen such that (1) they were reasonably different from 24 hours--over a two month period the short group would receive 65.5 days, and the long group 55.4 days, and (2) they were not so deviant from 24 hours as to cause the flies to break away from the external synchronizing

agent and maintain their own endogenous circadian rhythms (Bunning, 1967).

Flies were left in their respective light:dark boxes until death, except for counting and media changes. Vials were removed daily in order to count the number of flies remaining alive. Approximately every four days, flies were transferred to new vials containing fresh media.

The time of checking and transfer were determined by the light:dark cycles. All groups were checked at the same time so that death counts were made at the same time during the objective 24 hour day. Each group was checked only when it was in the light phase of the cycle. Obviously, disturbing the flies by bringing them into the light during the dark phase would be interfering with the purpose of the experiment, which used light to establish the rhythm. Additionally, it was necessary to check the flies at different times on different days. Otherwise it would be possible for the disturbance resulting from checking to act as a stimulus which would synchronize on a 24-hour schedule. By consulting a computer print out (see Appendix A) of the cycles, it was a simple matter to choose an hour for checking that met all the above conditions.

The only drawback for this method of checking was that occasionally there would be a day such that there was no time during which all three boxes were open, i.e., light-on condition, simultaneously. When this occurred,

the two boxes which were in phase were checked and the third box was not checked. A death count for the unchecked box was obtained by interpolating between the days previous and subsequent to the unchecked day. Choice of which box to leave unchecked was made in such a way as to insure that each box went unchecked an equal number of times. No box was unchecked more than one day in succession. The number of unchecked days for any given box did not exceed six for the whole experiment.

As noted in the subject section, the experiment was begun with all 1200 flies housed in vials and after two weeks half of these subjects (600) were transferred to bottles. The change was made for several reasons. First of all, checking the 240 vials daily was an extremely time consuming task. Using half-pint bottles and large numbers in each bottle cut down on the time and, more importantly, the amount of handling involved. Handling so many vials seemed to be causing a great deal of error variance. For example, a vial could slip and break during changing and thus 5 flies would be lost.[1] Secondly, five flies crawling over the small surface area afforded by exposed media in the vial caused the media to become soupy. This resulted in premature deaths (flies caught in media). By switching

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[1]During the course of the experiment several (less than 10) flies escaped. Once a vial broke, and a few times an individual fly escaped during transfer. These escapees were recorded as deaths.

half the flies to bottles there was additional time to make changes of vial housed flies into fresh vials more frequently. Thirdly, checking bottle-housed flies was very efficient. Flies were daily transferred into fresh bottles and dead flies (which usually remained stuck in the old bottle) were counted. And finally, the alternative housing method served as a comparison in order to get an indication whether or not one method was superior to the other. Because of the large number of Ss involved (1200), there were still ample numbers of flies (600) in each housing group to make statistically valid comparisons within a group.

RESULTS

The raw data consisted of number of deaths on each day for each cycle length group (see Appendix B). From the raw data, mean day of death was calculated for each sub-group, as shown in Table 3.

Table 3. Mean day of death for each group

	Vials			Bottles		
	Long	Normal	Short	Long	Normal	Short
Males	34.03	31.54	33.51	37.36	37.03	35.37
Females	49.49	48.04	50.56	48.15	43.44	41.34

To determine whether these means were significantly different a three way analysis of variance was computed. The analysis showed no significant effect of day length, which was the main variable of interest in the experiment. Of the other two variables (i.e., sex and housing) and their interactions, the only significant main effect was sex and the only significant interaction was housing by sex. The summary information for the analysis of variance is shown in Table 4.

Table 4. Summary of analysis of variance

Source	MS	df	F	P	Eta^2
Day length	621.82	2	2.30	N.S.	
Sex	43416.27	1	161.01	<.01	.1164
Housing	167.25	1	0.62	N.S.	
Housing x day length	652.90	2	2.42	N.S.	
Housing x sex	5564.21	1	20.64	<.01	.0149
Day length x sex	90.00	2	0.33	N.S.	
Housing x day length x sex	297.68	2	1.103	N.S.	
Error	269.95	1188			.859

Inspection of Table 3 shows that females are longer lived than males ($F = 161.01$, $p < .01$). The housing by sex interaction is such that males have a longer life span if they are housed in vials, whereas females live longer if housed in bottles. Table 5 shows mean life span of males and females for each housing condition.

Table 5. Mean life span for males and females in each housing group

	Vials	Bottles
Males	33.03	36.59
Females	49.36	44.31

DISCUSSION

The basic finding of this research is that there is no statistical evidence that cycle length has an effect on length of life for Drosophila. The only conclusion which can be drawn is that cycle lengths differing moderately from 24 hours are not deleterious to Drosophila as measured by longevity. This suggests that the organism has an adaptability during early life which allows him to adopt any cycle length within a certain range. Such an idea is provocative from a developmental point of view because previous research indicates that non-twenty-four hour cycles are harmful and/or organisms do not easily adapt to them; for example, the research cited in the introduction by Went (1962) on plants, or that by Lewis and Lobban (1957) on humans. The latter gave subjects day lengths of either 21 or 27 hours for several weeks. They found that some rhythms (e.g., temperature) adapted quickly, while others (e.g., excretory) were not completely adapted even after 6 weeks. Thus, there was a disassociation of rhythms.

Disassociated rhythms are usually assumed to be harmful on both empirical and theoretical grounds. Bunning (1967) gives a short review of research on disturbed phase relations which may result in disease, tumors, or working

incapacity. Practical questions in space and air travel have prompted research on disassociated rhythms. Humans who undergo transcontinental air flights, with their consequential phase shifts, show disassociated rhythms and behavioral impairment as measured by reaction time, decision time, a fatigue check list and critical flicker fusion (Hauty and Adams, 1966; Lewis and Lobban, 1957).

From the theoretical side, a number of theories of aging emphasize disassociations of functions. Mildvan and Strehler (1960) have developed a theory which hypothesizes that the various biological subsystems of an organism are subject to displacement as the result of stress. Energy is expended to restore the systems to balance. When stress surpasses the ability of the subsystems to readjust, the environment of other subsystems changes until they become incapacitated and death results. Similarly, Sacher (1956) has proposed a theory of aging which states that the mean physiological state declines at a constant rate over time. Random displacements occur, and when they extend below a certain limiting value death occurs. Weiss (1966) sees aging as a collary of development. The body accumulates a history of deviations with increasing disharmony of material relations and loss of integration until death results.

The conclusions drawn from both theoretical and empirical sources, then, are: (1) non-twenty-four hour cycling usually disrupts inter-rhythmic association, and

(2) rhythmic disassociation is harmful to the organism. In the present study, non-twenty-four hour cycles were introduced, but there was no adverse effect as measured by length of life. There are three reasonable possibilities which can explain this result:

1. The rhythms of the Drosophila were not disassociated at all because the abnormal cycling was introduced soon after emergence and the organism easily adapts at this stage.
2. The rhythms were initially disassociated but adapted soon enough so as to not result in any long term damaging effects.
3. The rhythms were continually disassociated, but there was no effect on longevity.

If the first possibility is occurring, it suggests an interesting area of research: the ontological development of rhythms. Although Drosophila are small it is still possible to study individual rhythms. Rensing (1966) has looked at the oxygen consumption rhythm and determined that it is in part genetically determined. An interesting question is: To what extent and at what stages of development can innate rhythms be modified without damage to the organism?

If the third possibility is occurring (disrupted cycling with no adverse effect), it would be surprising due

to the diverse research that suggests otherwise. If further research should show that non-twenty-four hour cycling disrupts rhythms without affecting longevity, it would throw serious doubt on theories which emphasize the displacement of subsystems as a major factor in aging.

Thus far the discussion has been concerned with the interrelation between rhythms when an organism is subject to non-twenty-four hour light regimes. An equally important issue is: What happens to one individual rhythm? It is possible that a rhythm could continue on a twenty-four-hour schedule, but generally it can be assumed that a rhythm will adopt the externally imposed cycling. A change in the timing of a rhythm also implies other changes in the rhythm. Using a sine wave as describing a theoretical physiological function, it is apparent that a change in frequency will result in changed wave length and perhaps changed amplitude. These parameters are important because they will describe any change in the "rate of living." For example, in a 30 day month, a fly on a 24 hour cycle experiences exactly 30 days, but one on a 22 hour cycle experiences 32.7 days. If we measure some function, say caloric intake, what will the differences, if any, be between the two flies?

Some theories of aging depend on a "wear and tear" concept of the accumulation of errors (for example, see Sacher, 1966, and Medveder, 1966). The organism wears out or accumulates error <u>through</u> time but <u>because of</u> use.

Amount of use can be measured by measuring physiological functions, such as caloric intake, as previously mentioned. If a method can be found which would alter "rate of living" or "use," it could allow a crucial test of these theories.

It was first assumed (Pearl, 1928) that shortened life span of Drosophila raised at high temperatures (originally reported by Loeb and Northrop, 1917) was due to an increased rate of living. Subsequent research has shown this interpretation to be wrong. Although flies raised at 25° C have a much shorter life span than those raised at 20° C, Maynard Smith (1963) has shown that flies transferred at half life from 25.5° C to 20° C live approximately as long as those continually raised at 20° C. Such experimental work supports a "threshold" theory in which aging proceeds at approximately the same rate in different environments, but death occurs earlier in a severe environment. Increased ambient temperature, then, is not an effective way to alter rate of living, although it is a stressful environmental change and can result in early death.

If earlier than normal age of death is a possible measure of stress, the experiment here suggests that changed frequency of day:night cycling is not stressful. The next question is: Has the rate of living been altered? This can only be determined by measuring specific physiological functions (all functions exhibit rhythmicity and many are circadian). It is likely that the rate of living was not

altered, e.g., that flies on a 22 hour schedule consume exactly the same amount of oxygen as those on a 24 hour schedule. However, if there are differences in the measured function it would throw serious doubt on wear and tear, and error theories. Any difference in the measured function between cycle length groups is a reflection of the amount of wear and tear which would occur, or amount of error which would accumulate.

In summary, then, the problems suggested by this thesis research are: (1) how are the interrelations among rhythms of an individual organism affected by altered day: night cycling; (2) does the developmental stage at which the alteration is introduced affect the organism's adaptation to it; and (3) is the altered cycling reflected in quantitative changes in specific rhythms?

A few minor points should be discussed. There were significant sex differences. Although there are often references in the literature to the effect that females appear longer-lived than males (Hollingsworth, 1967; Clarke and Maynard Smith, 1955), there has been little systematic study of the differences and no research on their cause. Like the other research, that presented here was not designed specifically to study sex differences, but it again confirmed that they exist.

The significant interaction between sex and housing is of interest. It suggests a variable which may contribute

to sex differences. Table 5 (p. 24) gives mean life span for males and females in each housing condition. It is apparent that males do better in bottles and females do better in vials.

While the experiment was not designed to uncover housing differences, I would like to speculate, based on informal observation, that an important variable is humidity level. It seems that relatively high humidity, found in vials, is beneficial to longer life. However, this is accompanied by sticky media. In the vials the small surface area breaks down quicker than in bottles where the surface area is greater. Male flies are smaller and become trapped in sticky media more easily than the larger females. What seems to be happening, then, is that for males the advantage of higher humidity in vials is overruled by the disadvantage of becoming stuck in the media. Hollingsworth (1967) mentioned this problem in discussing technical attempts to minimize accidental deaths. The solution to the housing problem, then, involves changing the vials often enough so that they will not become sticky, and changing the bottles often enough so that they will not become too dry.

Another problem is that the effect of housing differences, and any interaction concerning housing, may not be accurately reflected in the analyses of variance. This is because the bottle flies were not housed in bottles for the whole experiment. Therefore the true comparison is

between flies housed in vials, and flies housed in vials for two weeks and then housed in bottles. The differences between housing groups was insignificant and the problem mentioned here would tend to bias the results toward insignificance. The F value is very low, $F = 0.62$, and a value of 3.84 (d.f. = $1, \infty$) is required for significance at the .05 level. Therefore, it seems reasonable to assume that the results (no significant difference between housing groups) are valid despite the problem mentioned here.

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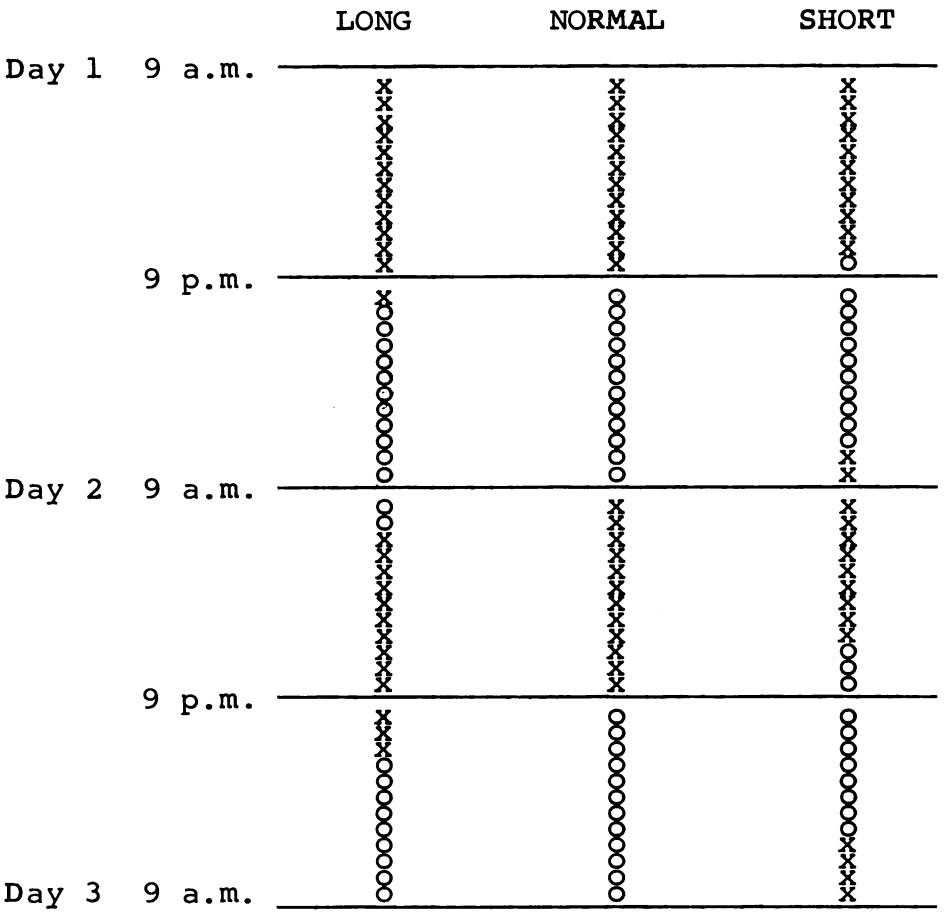
APPENDIX A

SAMPLE COMPUTER PRINT OUT OF LIGHT:DARK CYCLES

APPENDIX A

SAMPLE COMPUTER PRINT OUT OF LIGHT:DARK CYCLES



x's represent lights on condition and
o's represent lights off condition

Time of checking flies is determined by consulting the computer print out. For example, on day 2 checking could be done any time between 11 a.m. and 7 p.m.

APPENDIX B

RAW DATA

For each day length by housing conditions by sex group there are three columns: "day," "number dead," and "number remain." "Day" column gives number of days elapsed since the beginning of the experiment. "Number dead" column tells how many flies died on any given day. "Number remain" column gives both the number and percent (as the original total in each group was 100) of flies remaining on any given day.

FEMALES

Days	VIALS						BOTTLES					
	Short		Normal		Long		Short		Normal		Long	
	Number Dead	Number Remain	Number Dead	Number Remain	Number Dead	Number Remain	Number Dead	Number Remain	Number Dead	Number Remain	Number Dead	Number Remain
1	1	99	1	99	1	99	1	99	0	100	2	98
2	0	99	1	98	0	99	0	99	0	100	0	98
3	0	99	1	97	0	99	0	99	1	99	1	97
4	1	98	0	97	0	99	2	97	0	99	0	97
5	1	97	1	96	0	99	4	93	3	96	0	97
6	2	95	3	93	0	99	0	93	0	96	0	97
7	0	95	1	92	0	99	0	93	0	96	0	97
8	0	95	0	92	0	99	0	93	0	96	0	97
9	0	95	1	91	0	99	0	93	0	96	0	97
10	0	95	0	91	1	98	0	93	1	95	1	96
11	0	95	0	91	0	98	0	93	0	95	0	96
12	0	95	0	91	0	98	0	93	0	95	0	96
13	0	95	0	91	0	98	0	93	0	95	0	96
14	0	95	0	91	1	97	0	93	1	94	0	96
15	0	95	0	91	0	97	0	93	0	94	0	96
16	0	95	0	91	0	97	2	91	0	94	0	96
17	0	95	1	90	0	92	2	89	1	93	0	96
18	0	95	0	90	0	97	0	89	0	93	2	94
19	0	95	0	90	0	97	0	89	0	93	0	94
20	1	94	1	89	1	96	0	89	0	93	0	94
21	3	91	0	89	0	96	0	89	0	93	0	94
22	4	87	0	89	0	96	0	89	0	93	0	94
23	1	86	0	89	0	96	0	89	0	93	2	92
24	0	86	0	89	0	96	1	88	0	93	0	92
25	0	86	0	89	0	96	1	87	1	92	1	91
26	1	85	3	86	2	94	0	87	0	92	0	91
27	1	84	2	84	1	93	0	87	0	92	0	91
28	1	83	0	84	0	93	2	85	1	91	1	90
29	0	83	0	84	1	92	0	85	0	91	0	90
30	0	83	3	81	0	92	1	84	0	91	1	89
31	2	81	0	81	3	89	0	84	2	89	2	87
32	2	79	1	80	1	88	2	82	2	87	2	85
33	0	79	2	78	3	85	0	82	0	87	0	85
34	1	78	0	78	5	80	0	82	0	87	1	84
35	2	76	1	77	2	78	0	82	0	87	2	82
36	1	75	2	75	2	76	5	77	3	84	1	81
37	1	74	0	75	1	75	2	75	2	82	2	79
38	1	73	2	73	3	72	6	69	2	80	2	77
39	1	72	1	72	6	66	3	66	2	78	2	75
40	1	71	0	72	1	65	0	66	0	78	0	75

FEMALES--Continued

Days	VIALS						BOTTLES					
	Short		Normal		Long		Short		Normal		Long	
	Number Dead	Number Remain	Number Dead	Number Remain	Number Dead	Number Remain	Number Dead	Number Remain	Number Dead	Number Remain	Number Dead	Number Remain
41	1	70	1	71	3	62	3	63	2	76	0	75
42	0	70	1	70	0	62	6	57	4	72	2	73
43	0	70	2	68	0	62	0	57	6	66	1	72
44	2	68	1	67	2	60	9	48	6	60	2	70
45	0	68	4	63	0	60	4	44	6	54	1	69
46	1	67	4	59	1	59	1	43	4	50	1	68
47	1	66	1	58	1	58	2	41	7	43	2	66
48	0	66	1	57	0	58	6	35	9	34	4	62
49	1	65	1	56	6	52	10	25	2	32	0	62
50	3	62	0	56	2	50	6	19	6	26	3	59
51	3	59	2	54	2	48	5	14	7	19	7	52
52	2	57	2	52	1	47	5	9	6	13	6	46
53	2	55	4	48	2	45	0	9	5	8	12	34
54	1	54	2	46	0	45	1	8	1	7	1	33
55	1	53	0	46	1	44	0	8	3	4	5	28
56	3	50	2	44	2	42	1	7	2	2	1	27
57	1	49	1	43	4	38	0	7	1	1	2	25
58	2	47	2	41	3	35	0	7	1	0	3	22
59	0	47	1	40	1	34	2	5			2	20
60	6	41	4	36	5	29	0	5			3	17
61	7	34	8	28	4	25	0	5			2	15
62	6	28	3	25	1	24	1	4			3	12
63	1	27	3	22	1	23	1	3			2	10
64	5	22	1	20	2	21	1	2			1	9
65	0	22	2	18	3	18	0	2			0	9
66	0	22	3	15	2	16	1	1			1	8
67	3	19	4	8	3	13	0	1			1	7
68	4	15	3	5	2	11	1	0			1	6
69	2	13	5	0	2	9					2	4
70	4	9			1	8					1	3
71	2	7			2	6					1	2
72	1	6			2	4					1	1
73	1	5			2	2					1	0
74	1	4			2	0						
75	1	3										
76	0	3										
77	1	2										
78	0	2										
79	1	1										
80	1	0										

MALES

	VIALS						BOTTLES					
	Short		Normal		Long		Short		Normal		Long	
Days	Number Dead	Number Remain	Number Dead	Number Remain	Number Dead	Number Remain	Number Dead	Number Remain	Number Dead	Number Remain	Number Dead	Number Remain
1	0	100	0	100	0	100	1	99	0	100	1	99
2	0	100	0	100	0	100	0	99	0	100	0	99
3	0	100	0	100	1	99	0	99	0	100	0	99
4	1	99	2	98	2	97	2	97	1	99	2	97
5	2	97	2	96	2	95	4	92	2	97	2	95
6	3	94	5	91	3	92	5	88	0	97	0	95
7	0	94	0	91	0	92	0	88	1	96	0	95
8	1	93	1	90	1	91	1	87	2	94	4	91
9	2	91	2	88	2	89	5	82	2	92	2	89
10	1	90	2	86	2	87	2	80	1	91	1	88
11	1	89	1	85	0	87	0	80	0	91	0	88
12	1	88	0	85	0	87	1	79	2	89	0	88
13	1	87	3	82	2	85	2	77	3	86	0	88
14	0	87	1	81	1	84	0	77	1	85	1	87
15	0	87	0	81	0	84	1	76	0	85	2	85
16	2	85	2	79	0	84	1	75	1	84	0	85
17	2	83	3	76	2	82	0	75	5	79	0	85
18	0	83	1	75	4	78	0	75	0	79	1	84
19	0	83	1	74	0	78	0	75	3	76	1	83
20	3	80	0	74	1	77	2	73	0	76	0	83
21	6	74	7	67	2	75	0	73	0	76	2	81
22	6	68	5	62	1	74	0	73	5	71	0	81
23	1	67	1	61	1	73	3	70	0	71	1	80
24	2	65	2	59	0	73	2	68	0	71	1	79
25	1	64	1	58	1	72	1	67	0	71	0	79
26	1	63	2	56	1	71	0	67	0	71	0	79
27	1	62	0	56	3	68	0	67	0	71	1	78
28	3	59	1	55	2	66	0	67	0	71	3	75
29	0	59	1	54	0	66	1	66	1	70	0	75
30	1	58	4	50	0	66	0	66	0	70	0	75
31	1	57	0	50	1	65	2	64	4	66	7	68
32	1	56	1	49	1	64	2	62	5	61	7	61
33	4	52	1	48	6	58	1	61	0	61	2	59
34	0	52	0	48	7	51	1	60	1	60	1	58
35	1	51	1	47	1	50	3	57	1	59	0	58
36	3	48	2	45	1	49	0	57	1	58	3	55
37	2	46	0	45	3	46	0	57	2	56	0	55
38	9	37	9	36	6	40	1	56	1	55	2	53
39	9	28	9	27	6	34	0	56	0	55	1	52
40	0	28	1	26	2	32	2	54	1	54	1	51

MALES--Continued

Days	VIALS						BOTTLES					
	Short		Normal		Long		Short		Normal		Long	
	Number Dead	Number Remain	Number Dead	Number Remain	Number Dead	Number Remain	Number Dead	Number Remain	Number Dead	Number Remain	Number Dead	Number Remain
41	0	28	1	25	0	32	0	54	0	54	3	48
42	2	26	0	25	1	31	0	54	0	54	0	48
43	2	24	1	24	0	31	6	48	4	50	2	46
44	1	23	1	23	2	29	4	44	0	50	1	45
45	1	22	2	21	4	25	0	44	0	50	0	45
46	1	21	2	19	3	22	1	43	2	48	0	45
47	2	19	1	18	3	19	2	41	1	47	5	40
48	0	19	1	17	3	16	6	35	9	38	9	31
49	0	19	0	17	1	15	4	31	4	34	3	28
50	3	16	1	16	2	13	12	19	20	14	2	26
51	3	13	3	13	2	11	7	12	0	14	10	16
52	2	11	2	11	1	10	3	9	3	11	8	8
53	2	9	2	9	1	9	2	7	6	5	2	6
54	0	9	1	8	1	8	0	7	1	4	1	5
55	0	9	0	8	0	8	0	7	0	4	0	5
56	0	9	0	8	0	8	1	6	0	4	0	5
57	0	9	0	8	1	7	0	6	0	4	0	5
58	0	9	0	8	1	6	1	5	0	4	0	5
59	1	8	1	7	0	6	1	4	1	3	1	4
60	1	7	0	7	1	5	0	4	1	2	0	4
61	0	7	2	5	0	5	1	3	0	2	0	4
62	2	5	0	5	0	5	0	3	0	2	1	3
63	2	3	1	4	0	5	1	2	0	2	1	2
64	0	3	1	3	1	4	0	2	0	2	1	1
65	1	2	0	3	1	3	1	1	0	2	0	1
66	0	2	0	3	1	2	0	1	0	2	0	1
67	0	2	0	3	0	2	0	1	1	1	0	1
68	1	1	0	3	0	2	0	1	0	1	0	1
69	0	1	1	2	1	1	0	1	0	1	0	1
70	0	1	2	0	0	1	1	0	1	0	1	0
71	0	1			0	1						
72	0	1			1	0						
73	0	1										
74	0	1										
75	0	1										
76	0	1										
77	0	1										
78	0	1										
79	0	1										
80	0	1										
81	1	0										