THE DURATION OF LIFE OF *DROSOPHILA MELANOGASTER* IN VARIOUS ENVIRONMENTS

by

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ABSTRACT

A series of experiments were conducted to observe the effect of various environments on the duration of life of wild-type Drosophila melanogaster. Natural duration of life curves were prepared for these insects from 10.5 days of age and it was found that the average life expectancy of the males was greater than that of the females. A 10^{-4}M concentration of the mutagen acridine orange in the medium markedly reduced the average life span of Drosophila males and females. The 10^{-5}M concentration reduced the male life expectancy slightly but had no effect on the females. A method utilizing a separatory funnel type population container was developed to test the effect of a chemical stress on Drosophila populations. Some preliminary experiments were performed in order to evaluate the effect of 10^{-3}M 2, 4-dinitrophenol, a metabolic inhibitor, combined with various food environments on life duration. The sexes differed in their response to these environments and the relative durations of life of the flies were compatible with a priori considerations. From the results a non-linear relationship is suggested between the time of 50% population death and the rate of death. Starvation death curves were compiled for the Drosophila after they were exposed to various conditions which were thought to be representative of the natural environment. Following exposure to various media conditions, female Drosophila under starvation conditions outlived their male counterparts. The difference between the starvation life expectancies of the females and males tended to become greater after the insects had been kept under optimum conditions, due predominantly to an increase in female life duration. Utilizing a combination of a rapid 'quantitized' heat-shock and starvation conditions it was found that only the male starvation life duration was significantly decreased compared with the unshocked starved controls. The female starvation life duration did not appear to be significantly decreased by the heat-shock. Under starvation conditions wholly female populations had a life duration similar to female populations mixed with an equal number of males. Some explanation is given for the results obtained in this thesis and recommendations for further experiments are made.

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CONTENTS

A. INTRODUCTION

Transition from a living to a dead state is probably of great complexity from a physiochemical standpoint. It may be represented by an irreversible reaction ($S_1 \longrightarrow S_2$) which is a time-function process. In order to explain this transition many individuals have attempted to account for the actual physiological processes which ultimately culminate in the death of the organism.

From an experimental point of view it is more satisfactory to attempt to examine the phenomenon of death as a function of a single variable in the environment. In order to study the effect of the environmental variable on the death of a number of organisms we must define the population with which we are working. Two definitions are possible: (1) one can say that a population consists of many individuals whose innate physiological ability to maintain homeostasis differs from individual to individual about a mean value (Loeb and Northrop, 1917) or (2) one can say that all members of the population are alike; a different response is due to the inherent uncertainty in physiological function (Sacher, 1956, & Trucco 1961). According to the first definition heterogeneity of the population arising from certain genetical mechanisms and the inherent uncertainty present in development adds weight to the existence of a sensitivity parameter even in the highly inbred population. From view 2 it is reasoned that a statistical nature of mortality is inferred since the occurrence of death in a given time interval cannot be predicted for an individual however precisely the environment is controlled.

One can consider the organism as a physical system in which death occurs as the result of a fluctuation of physiological state exceeding the limits within which homeostatic regulation can occur. If a sensitivity parameter exists, the hardier organisms can withstand greater environmental fluctuations since they may have regulatory mechanisms which are capable of maintaining homeostasis more efficiently under these conditions or they may be capable of withstanding greater physiological fluctuations. The hardier organisms would therefore tend to live longer (view 1).

For an homogenous population the probility that in a time interval, t, a fluctuation will occur equal to or greater than a limit value, L, is identical for every member (view 2). Using n samples from the population, one could displace the mean physiological states from the pre-existing mean, m, to a series of different values m_1, m_i- - -m_n, for a time t by applying different stresses such as heat, chemicals, radiation and starvation whose strength may be represented as D_1, D_i- - -D_n. Then the fluctuation beyond L will be different for each sample group. These are representative of the probabilities of death so that a plot of P_i vs D_i is a dosage-survival curve. A time-mortality curve is produced when a population is subjected to an environmental stress and its rate of death recorded with time. Curves of the sigmoid form will theoretically result if the sensitivity parameter is distributed normally or if the fluctuations have the characterisitics of actual physiological processes (Sacher, 1956, Trucco 1961).

B. AGING THEORIES

Senility is a factor in the mortality of individuals and populations since adaptive capacities of most metazoans progressively decline with an increase in age. Generally speaking, aging theories fall into two classes. One class consists of the predestination type theory and the other, of the process type theories.

(a) Predestination

The genetical theory of mortality is a predestination type theory, asserting that the mortality curve is completely determined by the distribution of the genetical constitutions in the populations.

Since it has been observed that exposure to ionizing radiation accelerates the aging process, experiments to prove that mortality is caused by radiation-induced chromosome damage have been performed by many investigators. Lamy and Muller (1941) investigated the mortality of Drosophila folowing irradiation of early zygotes to determine whether it is caused by chromosome damage or gene mutation. They irradiated young embryos from triploid and diploid mothers. If mortality was of genetical origin the triploid offspring mortality rate should be different from the diploid rate. Also, a different mortality characteristic should be found for the 2 sexes. These genetical classes, however, did not yield different results. Lamy and Muller therefore concluded that most of the induced mortality and morphological abnormalities were probably caused by the effect of radiation on the cytoplasm of the treated cells. Since there is a large amount of maternally-pre-formed cytoplasmic organization to enable rapid development and differentiation of Drosophila embryos this assumption does not seem unreasonable. Muller (1959a, b) stated that he is unsure of this interpretation and the situation should be re-examined. He believes (Muller, 1940, 1950) that chromosome damage is the most probable cause of the aging effect of ionizing radiation.

Instead of using Drosophila embryos with their large content of maternally-pre-formed cytoplasm, Oster and Cicak (1958) have used many different larval stages which are active metabolically, principally anabolizing. Using the number that survived to the adult stage as a measure of mortality they found the males to be more susceptible to ionizing radiation than the females. This mortality difference may be

explained genetically, as the males have a haplo-X condition. Irradiating larvae just before pupation, Oster (1959) has used a more critical test in his work. Since ionizing radiation causes more ring-X chromosomes to be incapacitated than rods he has compared male larvae having a ring-X-chromosome with those having an ordinary rod-shaped-X. His tests showed those containing ring-X's are killed by radiation in greater numbers than those with rod-X's. Therefore it has been demonstrated that the mortality rate is increased by introducing a chromosome known to be more susceptible to damage. But there is still a possibility that the ring chromosome caused a cytoplasmic condition which could be the susceptibility factor. Part of the reason for mortality at this stage is that the pupal stage which follows the exposure is one of active cell proliferation and differantiation. The desoxyribose nucleic acid would be actively replicating. Any faults incurred in it by radiation could possibly manifest themselves throughout the remaining cell lineage. The adult Drosophila has little cell multiplication except that connected with reproduction and it can withstand radiation doses ten times greater than the pre-pupal stages. Muller (1959a) stated that radiation mortality in Drosophila represents basically the same phenomenon as in vertebrates, although mitotic divisions occurring all through the life-cycle of vertebrates cause death to be more evenly distributed. The so-called culminative nature of the aging effect of chronic radiation may be understood on a genetical basis since there is thought to be an absence of a threshold for radiation-incurred chromosome damage.

A number of theories of aging have postulated spontaneous somatic mutations as the primary cause. This is due at least in part to the aforementioned experiments which show that radiation has a life shortening effect and that this is probably due to chromosome damage. Failla (1958, 1960) stated that the life span is basically determined by the genetical constitution of the germ cells. He postulated that, on a genetical basis, aging could be attributed to the accumulation of spontaneous somatic mutations in the body cells. We therefore have a line of reasoning: (1) the rate of spontaneous mutation is determined by the stability of the genetical material and (2) the rate of aging is determined by the spontaneous mutation rate. This decrease in vitality due to spontaneous mutation of the somatic cells causes the characteristic

mortality rate and limits the life span.

In his genetical theory of aging Szilard (1959a, and 9b) is concerned with the period of human life between 70 and 90 years in which the majority of deaths occur. Szilard assumes: (1) the diploid organisms have 2n targets; (2) the hits are recessive and occur at random in space and time; (3) each individual begins life with a certain number of faults which are present in all somatic cells; (4) the random distribution of hits may be represented by the Poisson type of distribution; (5) a value f is assumed which is the surviving fraction of cells, and death occurs when the f falls below a critical value f*. Comparing chromosomal aberrations occurring in the liver cell of 2 strains of mice, one a shortlived and the other a long lived strain, Crowely and Curtis (1963) recently found the development of aberrations was inversely proportional to life span. This can be interpreted as experimental evidence in favor of the Szilard theory. Whether it is the aberrations which cause the aging or vice-versa is still to be proven.

(b) Process

It is stated in process theories that the aging process takes place according to a statistically regular law in all members of the population. The first process type theory is attributed to Gompertz (1825) who stated that the rate of mortality in humans tends to increase exponentially with age. Leob and Northrop (1917) and Brody (1923) both postulated that one could think of senescence and subsequent death as caused by a production and accumulation of some toxic substance which follows the law or monomolecular change in chemical reactions. Considering the distribution of physiological capacities in a population Simms (1942) pointed out that the exponentially rising mortality curve may be the result of only a linear decrease in these capacities. On this basis Sacher (1956) developed a stochastic theory of mortality. The two main tenants of this theory are: (1) an irreducible uncertainty in the mortality process which indirectly causes the variance in life tables; and (2) this irreducible uncertainty in the mortality process being attributable to the random physiological performances even under constant environmental conditions. At present the problem is to attempt to prove or disprove some of the theoretical considerations by experimentation.

C. INTRODUCTION TO SURVIVAL STUDIES OF DROSOPHILA

A study of the phenomenon of death may be done on many different levels (Table 1). From this table it is to be noted that the decreased survival capacity of the individual is the result of a hierarchy of events. Each level of organization and function is dependent on the preceeding level. Changes which end ultimately in death of the organism are generated at first by two primary processes described as stochastic and genetic. Stochastic refers to the fact that there is thought to be an element of indeterminancy in biological processes; therefore it follows that from a stress the degenerative events have associated with them certain probabilities of occurring. Each level must be taken into consideration. For example, at the molecular level, certain properties of macromolecules and their 'microenvironment' must be maintained so that the living system can continue to function. It is a well known fact that the functional properties of the protein molecule change with the hydrogen ion concentration. Changes in bonding properties causing age changes at other levels may be the result of some change in the hydrogen ion concerntration of the proteins' microenvironment with time. Utilizing the appropriate experimental approach, changes caused by aging and applied stresses could be elucidated for each level. In this thesis death at the population level will be of primary concern

Since survival studies with reference to the effect of temperature forms the most complete body of experimental work in Drosophila this aspect will be stressed in the introduction.

Loeb and Northrop (1917) experimented in an attempt to represent life span as a function of one variable-temperature. Loeb (1908) had already raised the question whether there was a definite temperature coefficient for the duration of life and whether this temperature coefficient was of the order of a chemical reaction. Determining duration of life at temperatures from 10-30°C for Drosophila melanogaster they found a Q_{10} comparable to that of a chemical reaction. On the basis of their experiments, they hypothesized life span was determined by the formation or disappearence of a chemical exhibiting the kinetics of a chemical reaction and that there is a definite temperature coefficient for life.

Alpatov and Pearl (1929) did research related to the influence of different temperatures on the duration of life of Drosophila genetically

and phenotypically similar and also genetically and phenotypically different. Plotting the length of life at different temperatures (18-28°C) they concluded that the duration of life is an exponential function of temperature. On calculating the temperature coefficients commonly used in biochemical and biological work they found a van't Hoff's Q_{10} value of approximately 2, which is the classical value for a purely chemical reaction in an homogenous system. This is also in agreement with Loeb and Northrop (1917). But great caution must be exercised in comparing biological processes with pure chemical reactions. From studies on developmental processes at various temperatures Alpatov and Pearl (1929) found the higher the temperature the smaller would be the dimensions of the resulting imago. Noting the two facts-shortening of life span and shortening of body dimensions with increasing temperature, they summarized their findings by saying that the important factor in determining the duration of life is the rate of energy expenditure during life.

Certain experiments (Maynard Smith, 1958; Clarke and Maynard Smith, 1961) have cast serious doubts on the hypothesis that the aging process follows the kinetics of a chemical reaction. Maynard Smith (1958) found that if young adult Drosophila flies were kept at 30°C for periods equal to about half their expectation of life at that temperature, and were then transferred to 20°C, their life span was equivalent to those flies kept continuously at 20°C. It may be readily seen that these results are not compatible with those that assume a continuous temperature dependent process. Clarke and Maynard-Smith (1961) explained the process on the following basis. They assume aging is approximately independent of temperature but as the age of the individual increases its physiological capabilities decline; that is, its vitality declines. If the vitality of the individual falls below a certain threshold appropriate to the temperature at which it is living the individual starts to die. At higher temperatures the level of vitality necessary for survival is greater than that for a lower temperature, so the differences in the rate of aging which that temperature causes in the individual, but to differences in the level of survival potential at different temperatures. On this basis they conclude that life span of Drosophila can be divided into two phases; (i) an irreversible 'aging process' whose rate is approximately independent of temperature from 15 to 30°C, and (ii) a 'dying process' which is initiated when aging has proceeded to a stage at which the

individual is no longer capable of maintaining a steady state at the temperature at which it is living, although it would be capable of maintaining a steady state at some lower temperature. Also the rate of the dying process is highly dependent on temperature, and can be reversed in flies transferred to a lower temperature (Clarke and Maynard Smith, 1961).

TABLE 1 (modified from Strehler 1959)

Different levels of aging and action of stresses

Level of Organization	Manifestation	Measure
POPULATION	increased probability of death	MORTALITY RATES
INDIVIDUAL	decreased adaptation	PERFORMANCE
SYSTEM AND ORGANS	decreased functional ability	PHYSIOLOGICAL TESTS
TISSUE	decreased functional ability	PHYSIOLOGICAL AND HISTOLOGICAL TESTS
CELLULAR	decreased function and increased probabilty of death	CELL PHYSIOLOGY BIOCHEMICAL CYTOLOGY
SUBCELLULAR ELEMENTS AND MACROMOLECULES	changes in molecular concentration arrangement and internal molecular structure	BIOCHEMICAL ANALYSIS OF MOLECULAR STRUCTURE PHYSICAL AND CHEMICAL PROPERTIES OF MACRO-MOLECULES

(A) INTRINSIC MOLECULAR STRUCTURE (BONDING CHANGES)

(B) RATE OF SYNTHESIS

(C) RATE OF UTILIZATION

(D) RATE OF TRANSPORT AND STORAGE (eg. precipitate)

CAUSE CHANGES IN:

GENETIC — STOCHASTIC

PRIMARY PROCESSES

D. EXPERIMENTS:

Part I. —— The natural life span and its modification.

Experiment No. 1 - Natural Aging

1a. Introduction

Natural survival curves of Drosophila have been of interest for many years. Among the first were those of Loeb and Northrop (1917), followed a few years later by those of Pearl (1921) and Pearl and Parker (1921, 1922a, 1922b, 1922c, 1923). Recently, Maynard Smith (1959) and Strehler (1961, 1962a&b) have renewed the interest in these studies.

In order to establish natural survival curves for the Drosophila stock which was to be used in the present series of experiments an experiment was performed to determine the effect of natural death on population size from the age of 10.5 days. This age was chosen for a number of reasons: (1) the flies first appear to decline in their vitality at approximately this age; (2) the egglaying and, hence, reproductive potential, of the flies begins to fall so that it is much easier to keep a constant environment free from many larvae and a massive accumulation of eggs even after one day; and (3) the number of deaths before this time is usually small (10-15%) although the stickiness of the media (due to larvae) may increase it slightly.

1b. Materials and Methods

Progeny from one-to-six-day-old parents of a moderately inbred wild type Drosophila melanogaster stock were used. A population of equal numbers of males and females was allowed to lay eggs for a period of less than one day on an agar-cornmeal-molasses medium (medium No. 1, appendix) in half-pint milk bottles placed in the incubator. Offspring of the same patents hatched within one day in the same half-pint milk bottle were mildly etherized at the mean age of 10.5 days. The Drosophila were divided into two approximately equal groups (each containing both sexes) and placed in separate half-pint milk bottles containing the same ingredients as medium No. 1, but with the omission of the live yeast(medium No. 2). Since Pearl and Parker (1927) found that there was a relationship between density of population and life duration the population densities were

kept within certain limits. They found that at a density of 15 flies per oz. the mean life duration was 38 days and at 35 flies per oz. it was 39 days for Drosophila populations of equal numbers of each sex. In the following experiments the densities are kept within these limits.

A note on the environment would now be useful. Throughout the experiments the incubator was kept in the Biological Science building sub-basement which is below ground level. Although the temperature was relatively constant, 25.5 $\pm$ 1°C, on a few occasions it did rise to 26 $\pm$ 1.5°C. Perhaps more important was the fluctuation in relative humidity. Although the air was kept 'fairly saturated' using a large pan of water at the bottom of the incubator the degree of saturation on the inside of the incubator was also influenced to some degree by that outside, which is a function of the weather conditions. Using a recording hydrothermograph the relative humidity was found to vary between 55-75% inside the incubator. It was not possible to measure the humidity inside the cotton batten plugged bottles, but it probably differed from that outside.

The populations were transferred to fresh media bottles at least every five days (Pearl and Parker 1922a) until the population was depleted by 50% of the original number. Each time the medium was changed the flies which had died in the interval were counted and tabulated according to sex. After all the flies from both populations had died the results were tabulated and a conventional plot of percentage living versus time in days was made. Respective times of death for 50% of the populations were obtained from the graphs.

1c. Results and Discussion

TABLE 2

Comparison of the natural aging of Drosophila males and females

	MALES		FEMALES	
Trial	n	50% death time	n	50% death time
1a	47	33.9 days	50	30.1 days
1b	62	38.2 days	62	30.4 days

The results are presented in Table 2 and Figures 1a and 1b. In both trials it is apparent that on the average the males live longer than the females. In trial 1a the difference is 3.8 days for 50% population decrease in favor of the males and in 1b it is 7.8 days again in favor of the males. The differences between the two trials is also consistent with the statement that in the stock of Drosophila malanogaster used the males are generally longer living than the females. In Pearl's experiments (as noted in sect. 1b) the females lived longer than the males but Maynard Smith(1959) found that longevity may be in favor of either sex depending on sex-limited genes.

Experiment No. 2 - Modified life span

2a. Introduction

In the thesis introduction it was stated that a possible cause of aging is the accumulation of somatic mutations. Investigators have further substantiated this hypothesis by the discovery that ionizing radiation has a life-shortening effect. By analogy one could expect that chemical mutagens may produce similar life-shortening effects. Auerbach and Robson (1944) were the first to demonstrate a chemical mutagen whose potency was comparable to ionizing radiation. This was nitrogen mustard. Since the discovery that mustard gas was a mutagenic agent many related chemicals were tested and subsequently many of these were also found to possess similar activity. It is difficult to differentiate between the action of ionizing radiation and that of chemical mutagens. Chemicals which were thought to produce mutations as an indirect result of radiation were directly applied to the cell with successful mutagenic results (Dickey, Cleland and Lotz, 1949). Peroxides were thus found to be chemical mutagenic agents and may be termed radiomimetic in respect to their action. Rapopart (1948) first investigated the aldehydes with special preference given to formaldehyde, which was found to be a very potent mutagenic agent under certain conditions. Oehlkers (1953) discovered that urethan causes chromosome breaks in Oenothera and Vogt (1950) induced mutations in Drosophila using this chemical.

Bases such as acridine orange and pyronin have induced mutations in Drosophila (Clark 1953). Acridine orange is thought to owe its mutagenic activity to its capacity of combining with the acid groups of desoxyribosenucleic acid and so preventing the association of nucleic acids and protein at the time of chromosome duplication. Muller (1959a) stated, that site mutation is also a possibility. That is, a mutagenic agent may inhibit the physiological capacity of a gene by combining with it. Adenylic acid binds acridine orange (Steiner and Beers, 1958) and it also aggregates in a reversible reaction with native desoxyribosenucleic acid (Bradley and Felsenfield, 1959). A photodynamic action is also characteristic of acridine orange (Tennent, 1942). Karreman et al (1957) found that acridine orange exhibits competitive inhibition with respect to adenosine triphosphate for sites on animal muscle 'in vitro'. The advantage of using acridine orange to modify aging is related to its mutagenic quality since it could cause life-shortening by induced somatic mutation. As noted, however, it has other degenerative physiological properties which may obscure its mutagenic activity.

2b. Materials and Methods

Since acridine orange may react with nucleic acid the yeast and cornmeal were omitted from medium No. 1 (see appendix Medium No. 3), all other quantities remaining the same. 2cc. of 10^{-3}M acridine orange and 10^{-4} acridine orange were added to 18cc of liquid media (at 55-60°C) so that concentrations of 10^{-4}M and 10^{-5}M media were obtained (appendix Media Nos. 4 and 5). Some bottles were maintained as 'normal' rather than 'control' since they consisted of plain medium with nothing added. The slight variation was not regarded as a limiting factor.

The bottles were made up in pairs so that there was one for each sex for each trial concentration used. Drosophila were prepared according to the method described previously in 1b. At the mean age of 12.5 days the insects were etherized and segregated according to sex. They were then placed in their respective media bottles (only one sex-type in any one bottle) which were kept in an enclosed cardboard container at all times except briefly during inspection and transfer. The inspection and transfer was done under a dim electric light in a

windowless room. These precautions were taken to reduce the photodynamic effect of acridine orange to a minimum.

2c. Results and Discussion

TABLE 3

The effect of acridine orange on Drosophila life duration

Acridine orange concentration	MALES		FEMALES	
	n	50% death time	n	50% death time
10^{-4} Moles	66	22.8 days	67	20.3 days
10^{-5} Moles	58	48.2 days	82	44.4 days
normal	46	58.0 days	47	40.5 days

The results of the experiment are presented in Table 3 and Figures 2a and 2b. The 10^{-4}M acridine orange trial showed a pronounced effect of the chemical agent. The male population had decreased 50% by the time the population had reached a mean age of 22.8 days. By comparison with the normal it had 35.2 days of potential life left. The female population was depleted by 50% at a mean age of 20.3 days, with 20.2 days of potential life left under normal conditions. At 10^{-5}M the situation was very different. The reduction of the concentration by ten appears to be critical since the male population almost approached normal and the experimental female population appeared equal to the normal one with respect to life expectancy.

On the basis of this individual experiment it is very difficult to explain the results by a specific mechanism of action of the acridine orange. Since the mutation process is hypothesized to be without a threshold it may be most meaningful to consider the lowest effective concentration. The fact that the male population was reduced in life expectancy at the 10^{-5}M level whereas the female one was not may be explained on a genetical basis. A mutation occurring on one X-chromosome of the female Drosophila would not necessarily stop the physiological capacities of that gene locus-type activity since there are two X-chromosomes in each female cell. In the male a mutation would

likely cause degenerate changes in the cell if it occurred on the single X-chromosome since there is only one locus present. It is also possible that there has been a modification of life span from the non-genetical factors discussed previously. Further analysis of the data using probits is being considered. Lastly, it is again observed that under 'normal' conditions the males were found to live significantly longer than the females. The fact that the media was different and the sexes were segregated appeared to have no influence on this fact although the possibility exists that the total life span of the flies is different than in Experiment No. 1.

Pearl and Parker (1922a) found that within a moderately inbred line of Drosophila melanogaster genetic differences in duration of life remain constant over 10 to 25 generations, so that a comparison of Experiment No. 1 with Experiment No. 2 is warranted. In Experiment No. 1 the 50% population death time occurred at the average time of 36 days for the males and 30.3 days for the females, whereas in Experiment No. 2 the corresponding time for 50% death was 58 days for the males and 40.5 days for the females. Further experiments are indicated to determine the environmental variable which is the basis for the differences observed.

Part II. —— Death as a function of the application of extreme environmental stresses.

Experiment No. 3 - Death by a combination of foods and 2, 4-dinitrophenol

3a Introduction

Death results from failure of the organism to maintain its homeostasis whether it is produced by aging or by the action of some extreme environmental stress. Dobzhansky (1957) describes death as a stage at which the organism has lost its capacity to maintain a steady state. Certain environmental variables may be applied to Drosophila and the life curves obtained may give an insight into the maintenance of its internal environment.

The ability of 2, 4-dinitrophenol (DNP) to block oxidative phosphorylation is the mechanism of toxication. This chemical uncouples the oxidation and phosphorylation process. Inhibition of formation of

high energy phosphate bonds prevents the utilization of the energy provided by respiration and glycolysis. An increase of the respiration rate is the most characteristic preliminary effect of the dinitrophenols. Bodine and Boell (1936, 1938) found that the application of 2.5 times 10^{-4} M of DNP to embryos of Melanoplus differentialis increased oxygen uptake to three times normal. This increase in respiration rate was attributed to the increased availability of the orthophosphate and the adenylic acid phosphate acceptors which limit carbohydrate metabolism under normal conditions (Judah and Williams-Ashman, 1951). Using isolated mitochondria Judah (1951) showed that DNP in concentrations of about 10^{-4} M inhibits the oxidation of pyruvate and this inhibition can be reversed by adenosine triphosphate, coenzyme I, and added L-malate. All of these are important factors in the metabolic pathway by which carbohydrates are utilized for the production of energy in living systems.

Wilson and King (1955) experimented with the effect of 10^{-3} M DNP on the uptake of radioactive phosphorus in Drosophila. Although they give no life curves they state that freshly eclosed males died after 24-48 hours feeding on 10^{-3} M DNP while none lived to 72 hours. Many females survived as long as 72 hours. Wilson and King did not expect this since treated males incorporated more exogenous phosphorus into their tissues after the feeding period of 26-32 hours than did DNP-treated females. The situation was reversed in the controls. Wilson and King were puzzled by this lower tolerance of males than females to DNP.

From the foregoing brief review one can see how useful DNP could be in studying homeostatic regulation. Administration of DNP can drastically affect the amount of utilizable energy in the organism. Decrease in energy will limit regulation since regulation and continued life require a continual energy supply. Strehler (1962b) states that the appropriate dimensions of physiological functions are work output per unit time. Death will occur when the energy is not available to maintain and regulate the organism's processes.

It was decided to perform several preliminary experiments to examine some of the relationships between 10^{-3} M DNP concentration and various food environments. These environments should be viewed as extreme stresses - the more extreme the stress the lower the duration of life of the stressed population should be. Sternburg (1963) views all

stress phenomena in insects as having a common basis. Whether the stress in question is chemical (eg. DNP) or physical (eg. stress paralysis) the symptoms may be related to abnormal secretions of physiologically active substances. That is, abnormal hormone release may cause profound pathological consequences.

3b. Materials and Methods.

Drosophila were prepared as previously described (1b). In order to measure the progressive death of the populations tested the author devised a separatory funnel population container consisting of an extruded glass rod with a glass wool wick, a four-ply layer of gauze with a metal ring in the middle and an ungreased stopcock at the bottom (Fig. 3a). The liquid medium was injected into the extruded glass tube with a hypodermic. Death counts were obtained by opening the stopcock of the funnel at specific time intervals. This setup was advantageous for two reasons: (1) the dead flies are continuously removed from the population; (2) if one requires examination of the insects immediately after death this is possible. One can therefore keep all the flies in one container and still retain the aforementioned advantages.

The first run utilized 10^{-3}M DNP media as described in the appendix (Media No. 6). Basically it consisted of 10^{-3}M DNP, 1% fructose, a few drops of dead yeast on the wick and a salt mixture. A control was run with no DNP. Insects 5-6 days old were etherized and placed in individual 500ml. population containers. For the second experiment flies 3-5 days old were etherized and placed in 500ml. separatory funnel population containers. These were both fed media containing 10^{-3}M DNP but one had 1% fructose with no yeast (see appendix Media No. 7) and the other 4% fructose with no yeast added (see appendix Media No. 8). Since this experiment was conducted over a period of 300 hours it was not possible to take readings every one or two hours. Readings, however, were taken at intervals necessary to construct reliable life curves.

3c. Results and Discussion

TABLE 4

Effect of DNP and Various food environments on Drosophila death rate

	n	20-80LSS[1]	50% dead	n	20-80LSS	50% dead
(1) 10^{-3}M DNP 1% fructose yeast	73	-0.09P/HR	19.0 HR	51	-0.201P/HR	17.2 HR
(2) 10^{-3}M DNP 1% fructose no yeast	45	-0.044P/HR	27.1 HR	75	-0.022P/HR	51.0 HR
(3) 10^{-3}M DNP 4% fructose no yeast	46	-0.0376P/HR	68.5 HR	71	-0.0048P/HR	143.0 HR

[1]20-80LSS refers to the Least Squares Slope of the life curve calculated between 20 and 80% of the population deaths. Its rate is measured in proportions per hour; that is, the total population is 1.

The results of the experiments are given in Table 4 and Figures 3b and 3c. Life curves for the control in (1) were not taken but the author found that the control population lived considerably longer than the experimental one. In the second experiment represented by results (2) and (3) the two were compared with themselves; no absolute control was utilized. Certain results stand out in Table 4. First, there was a sexual dimorphism with respect to the effect of the three different environments. In population No. 1 males had a 50% population death point at 19 hours whereas 50% of the females died at the 17.2 hour mark. This is suggestive that under these conditions the results of Wilson and King (1955) on the rate of phosphorous uptake and its relationship to DNP tolerance are vindicated. The death-rate was faster for the females (-0.2P/HR) than the males (-.09P/HR). In population No. 2 it is interesting to note the results are very much like those of Wilson and King (1955) as quoted in 3a. The females now outlived the males and also died at a slower rate as can be noted in Table 4. In population No. 3 which was run concurrently with population No. 2 the differences became more pronounced. Males now had a time of 68.5 hours for the death of 50% of the population and females a time of 143 hours.

The slopes are also different (Fig. 3c). The male slope at -0.037P/HR is very much higher than that of the females (-0.0048P/HR). It is, however, not correct to compare the slopes on this basis since if one population lives a great deal longer than the other it will have more latitude in which to die. The relationship between the time of 50% population death and the slope was plotted (Fig. 3c). It is evident that under the various stresses there is not a linear relationship between the increase in life span and the rate of death. Prior to the time of 42 hours for 50% death of the population the females have a greater death rate than males. After the 42 hour mark it may be seen that the male death rate for the same 50% time of death as the female population becomes greater. This points out a sexual dimorphism with respect to the rate of death as well as the time of death for the three environments to which the Drosophila were exposed.

The populations may be said to exhibit relative death times which are compatible with a priori expectations. Population No. 1 was probably more active due to the presence of yeast in the medium and therefore its energy requirements were greater than population No. 2. The effect of DNP would therefore be more pronounced. Due to its greater food concentration (4% vs 1%) population No. 3 required less volume of media. Its uptake of DNP was therefore less as judged by the quantity of excretions on the flasks (much greater in the 1% fructose flasks). Since no absolute control was present for trials 2 and 3 it is not known whether the food would be a limiting factor by itself under the testing conditions.

Experiment No. 4 - The effect of starvation

4a. Introduction

Pearl and Parker (1924) carried out an extensive study on the influence of complete starvation from birth on the duration of life of Drosophila melanogaster. It was their belief that using complete starvation from birth as an experimental tool they could analyse the 'innate protoplasmic and constitutional' differences among individuals. 'Inherent vitality' was the terminology they gave to the energy with which an organism was born or hatched. They found that females lived

longer under complete starvation and exhibited a higher absolute variability than males. From an experimental point of view starvation experiments are valuable since they may point out fundamental facts about regulation and mechanisms of death. Compared with aging experiments, starvation experiments are also relatively short in duration and the information once accumulated can be utilized to plan more experiments. Starvation curves are of ecological importance if one is interested in the feeding habits of an organism.

4b. Material and Methods.

Drosophila were prepared as in 1b. Starvation replication tests were done using one bottle of insects which were 0-1 days old. The males replication tests were done in a pair of 2000ml. flasks and the females in a pair of 1000ml. flasks. On etherization, the flies were segregated according to sex and placed in their respective flasks in the incubator under complete starvation. Complete starvation is defined as the absence of food and water. To obtain this condition the separatory funnel was merely plugged with a layer of four-ply gauze; the feeding tube was excluded. Readings were taken by opening the stopcock and letting the dead flies fall into a test tube for counting. A small flashlight beam was directed down the stopcock opening from above so that only the dead ones were released. These results were recorded every one or two hours and a set of duration of life tables were constructed.

Since the sexes were placed in different flasks further experiments were conducted. A series of starvation experiments were conducted so that the sexes from one population of Drosophila (flies hatched from the same bottle) were placed in the same size container making possible a comparison of life duration between the sexes. Two sets of experiments were done. One utilized insects from populations picked at random from the incubator; the other made use of insects from different populations put under controlled conditions. The media of flies under controlled conditions was kept fresh.

4c. Results and Discussion.

TABLE 5

Starvation replication tests

	n	50% death time	n	50% death time
MALES	63	17.6 HR	54	17.3 HR
FEMALES	31	19.0 HR	19	18.8 HR

In the first set of experiments times of 17.6 hours and 17.3 hours were obtained as replicate values for the death of 50% of the male population while times of 19.0 hours and 18.8 hours were obtained for the females (table 5). Starvation death curves are presented for this experiement in Figs. 4a, 4b, 4c, and 4d. It is evident from these values that replication of results using the method developed is very good.

TABLE 6

Starvation of Drosophila populations picked at random from the incubator

Age	MALES		FEMALES	
	n	50% death time	n	50% death time
5-6 days	26	11.9 hours	23	12.6 hours
4-6 days	86	16.0 hours	92	17.6 hours
1-2 days	132	27.7 hours	113	31.5 hours

TABLE 7

Starvation of Drosophila populations raised on fresh media

Age	MALES		FEMALES	
	n	50% death time	n	50% death time
3-4 days	74	24.8 hours	74	37.8 hours
2-3 days	55	14.7 hours	54	30.8 hours
1-2 days	20	25.6 hours	45	35.8 hours
1-2 days	62	32.0 hours	50	38.0 hours

The values for starvationdeath of Drosophila populations picked at random from the incubator are given in Table 6 and starvation death curves, in Figure 4e. The values for starvation death of populations raised under controlled conditions and subjected to starvation are given in Table 7. In both experiments the females consistently live longer than the males. On comparing the values for the randomly picked populations with those of the control populations it is apparent that the difference between the sexes is greater in the controls. This difference is due to the increase in duration of the life of the females. This is expected since under control conditions the females would tend to manufacture and store more eggs and enlarge associated structures. During starvation reabsorption is possible to some degree. Pearl and Parker (1924) compared the fact that females outlived males under starvation conditions with their finding that females also outlived males under natural feeding conditions. They thought that death by starvation was a measure of some innate energy factor and was related to natural death. Since the strain of flies used in our experiment gave higher life span values for males this idea propounded by Pearl appears invalid. It would, however, be interesting to obtain starvation death curves at higher ages of Drosophila.

Experiment No. 5 - Starvation and heat-shock

5a. Introduction

Temperature extremes have many diverse effects on living systems. Drosophila are poikilotherms since they may be considered to respond passively to environmental temperatures, from a regulatory point of view. Insects, however, may achieve slight regulation against temperature stress by altering water loss and retention. Also, insects generate considerable heat particularly in their flight muscles. Since the temperature of an organism depends on the balance between heat gained and heat lost, the temperature of the environment cannot absolutely be regarded as the temperature of the insect.

For a given species the temperature effects depend mainly on the level and duration of exposure, the thermal history and the rate of temperature change. The debilitating effects of extreme

temperatures are the result of numberous mechanisms. An increase in temperature will cause a change in activity of enzymes which may put key reaction sequences out of balance. Enzymes may become inactive and denatured above certain temperatures. Lipids may change their physical properties thus causing changes in membrane permeability. Release of toxic substances due to heat stress may cause pathological changes. In insects water balance is of prime importance at elevated temperatures due to the structure of the trachea which permit rapid dessication.

Although much experimental work has been done on the influence of various temperatures on poikilotherms little work can be found on the influence of a rapid quantitized temperature change. By 'quantitized' one means that the exact slope of the temperature increase and decrease is given so that reproducibility of experimental conditions is ensured. Strehler (1961, 1962) took Drosophila and "exposed them for variable periods of time to high temperatures by immersing the lower three-fourths of the tubes in a constant temperature bath for the prescribed period...." He did not take into account the fact that difficulties arise when someone tries to reproduce these experiments. A knowledge of the rate of heating and cooling of the environment would contribute to a further understanding of any observations since the reaction of living systems to an environmental change depends not only on the magnitude of the change but also on the rate of change.

5b. Materials and Methods.

Drosophila were used as prepared in 1b. They were removed from their half-pint milk bottle container, lightly etherized, and roughly divided into two equal parts of mixed sexes. Both groups were placed in 2 X 15 cm. test tubes, one maintained at 26 $\pm$ 1 ^{o}C, the other placed into a circular Warburg constant temperature bath (Precision Scientific Co.) This tube was immersed in the water so that only 1 cm. was above water and a cotton batten plug inserted so that there was 3cm. of space for the insects in the extreme bottom of the test tube. The control tube was similarily plugged. A third test tube with a thermometer inserted through the cotton plug was placed in the water bath. Readings recorded from a thermometer placed directly in the water bath were compared with those of the thermometer in the test tube. It was found that the readings did not differ more than 0.5^{o}C

during the period of temperature changes. The readings taken directly from the water bath were used in the temperature chart. One-half hour after etherization (all the flies were now fully active) the auxillary heating coil on the Warburg was deployed. A heating curve of almost constant slope was obtained. When the temperature of the water bath reached 38°C the heat was turned off and a cooling coil connected to the cold water tap was engaged. The cooling coil, which was constructed by the author, consisted of 15ft. of 1cm. aluminum tubing coiled into loops of 10cm. in diameter with connecting parts of rubber tubing. The coiled section was fitted around the bottom extremities of the Warburg in a large semi-circle. To keep the cooling slope relatively constant required practice. Continual time-temperature readings were taken and graphed immediately. After the temperature reverted to normal, the control and experimental tube contents were placed into separate 500ml. separatory flasks as in the starvation experiments and observations recorded. An additional experiment utilizing a mixed population and a completely female population under starvation conditions only was also performed.

5c. Results and discussion.

TABLE 8

Heat-shock and starvation

TRIAL	MALES				FEMALES			
	SHOCKED		CONTROL		SHOCKED		CONTROL	
	n	50% dead	n	50% dead	n	50% dead	n	50% dead
1. July 14'63 1-2 days old	57	25.8HR	62	32.0HR	57	43.7HR	50	38.0HR
2. July 17'63 3-4 days old	87	19.1HR	74	24.8HR	84	37.6HR	74	37.8HR
3. July 31'63 1-2 days old	60	22.2HR	20	25.6HR	45	35.1HR	55	35.8HR

On the basis of the data in Table 8 and a graph of one of the trials presented in Figure 5a an evaluation of the effect of the heat-shock can be attempted. Trial No. 1 shows that the 50% death time of the population of shocked males was 25.8 hours compared with 32.0 hours for

the control. This suggests that the males are significantly affected by the temperature changes administered. This was substantiated in trials 2 and 3. On the basis of the time of 50% population death in trial 1 the shocked females outlived the control female population. This result was not reproduced in the subsequent trials. In fact it appears that there is no significant difference between the shocked females and their controls. At first this was thought to be a logical outcome of mixing. The organisms were mixed so that in any one group both the shocked females and shocked males would be in the same flask since there is the possiblity that the eggs laid by the females or other secretions of one sex may be edible. The disparity in reaction of the sexes was thought to be due to mixing since the shocked males died first and left shocked females in a total female environment whereas the control males were still living with the control females. It was thought, then, that the absence of the males could decrease the metabolism or activity of the shocked females and that was the reason they lived as long as the unshocked controls. The results of a test designed to show if there is a mixing effect of the sexes on the starvation experiments are given in Table 9.

TABLE 9

The effect of mixing and starvation

MIXED				UNMIXED	
	MALES		FEMALES		FEMALES
n	50% dead	n	50% dead	n	50% dead
55	14.7HR	54	30.8HR	77	30.5HR

It can be seen that unmixed females do not live longer under starvation conditions than their mixed counterparts, (~~the mixed counterparts~~) the mixed females having a 50% death value of 30.8 hours and the unmixed females, a value of 30.5 hours. There appears to be no significant difference between the duration of life of the mixed females

and the unmixed females.

The aforementioned experiments point out a difference between the sexes in their reaction under starvation conditions. Maynard Smith (1957) found that exposing adult Drosophila subobscura females to a temperature of 30.5°C for a period of 5-12 days increased their life span up to 50% over the controls. There was no effect on the male life span when similarily exposed. Since the exposure to 30.5°C for that time reduced the egg laying capacity of the females but not the sexual capacity of the males he concluded that this temperature increased the life of the females by reducing reproductive activity. He also exposed females for 45 minutes at 33.5°C (allowing 10 minutes for the temperature to change from 20°C but with sudden temperature loss), which is about half their mean survival time at that temperature, and then kept them for 3 hours at 20°C with drinking water. These females lived significantly longer than the controls at 20°C when both were subsequently exposed to 33.5°C. A group of females kept at 20°C in dry air for 18 hours then for 3 hours with water also survived longer than the controls when exposed to 33.5°C. Smith postulated that over-drinking may be the reason heat death is slower in the groups described, since death may result from dessication of the fly at 33.5°C. He also noted that the exposure to 33.5°C increased the activity of the flies at first but after an exposure of 50 minutes the active period was followed by a sluggish period. At high temperatures insects have a characteristic heat coma point depending on their thermal history (Mellanby, 1954). When the heat coma point is reached the organism goes into a stupor. In this author's experiments it is possible that the decrease in the metabolism of the females due to the high temperature exposure may compensate for any deleterious effects of the heat-shock, whereas the metabolic rate of the males is not affected enough to compensate for the effect of the heat-shock. Also since the fat and water reserves of the females are greater than the males any change in them would tend to be more pronounced and therefore more significant in the males. In any case it has been demonstrated in these preliminary experiments that the starvation method for the determination of the effect of an environmental stress is workable.

E. GENERAL DISCUSSION

It has been demonstrated in this thesis (Experiment 1) that aging is the limiting factor in the duration of life of Drosophila melanogaster under near-natural conditions since there was a decline in vitality following the age of approximately 10 days. The length of life of Drosophila in its natural environment is important in the dynamics of populations as the rates of death and reproduction must be approximately the same in order to maintain a population of constant size. The influence of aging on the death rate is a dynamic balance of many evolutionary factors. Among these factors one must consider the physiological limitations of the organism, the reproductive cycle, the selective advantages gained by segregation and recombination at reproduction, and environmental stress. If a group of organisms is placed under a stress the life duration of the population will be altered depending on the type and strength of the stress. Aging may be modified by a long-term stress such as the mutagen, acridine orange. Acridine orange (Experiment 2) caused a significant life-shortening effect on D. melanogaster. Whether or not the stress of introducing this chemical into the environment affected the natural aging process is not known.

Harsher short-term stresses-the metabolic inhibitor 2, 4-dinitrophenol, starvation, and heat-shock (Experiments 3, 4, and 5)-shortened the life duration by upsetting the normal regulatory mechanisms to such a degree that the organisms could not maintain homeostasis. Under starvation conditions females outlived the males, a reversal of the situation in aging experiments where males outlived the females. The use of heat-shock and starvation significantly affected only the male life duration. These results might be explained by the fact that the females have larger reserves of water and fat. Evidently the females have physiological mechanisms which tend to make them hardier under the short term stresses of heat-shock and starvation.

F. RECOMMENDATIONS FOR FURTHER WORK

A number of experimental variables which may prove helpful in evaluating an effective approach to life duration studies are presented in Table 10. It is not all inclusive but contains many of the variables which must be considered in the experimental design. The number of variables and their combinations are such that one must choose the appropriate experimental approach by considereing the time available and the possible level of importance of the results. Of foremost importance is the elucidation of the basic principles which underlie the process in question-namely duration of life. A study of the effect of chemical and physical mutagenic agents on the life span is a field which holds many unexplored possibilities. It would be particularily interesting to perform experiments on chronic exposure of successive generations to low doses of chemical mutagenic agents. Determination of the effect of specific metabolic poisons on the death rate may give some insight into the energy required for the operation of the regulatory process. Performing this type of experiment on populations of Drosophila at different ages could help elucidate the decline in regulatory capacity. The 'quantitized' heat shock may be administered more than once and at different intensities than those described. It should be possible to measure a difference in reaction to heat shock at different ages.

TABLE 10

Experimental variables in aging studies

TIME (1) Duration of treatment:

(a) Short exposure 'quantitized' temperature shock
- known concentration of chemical agent

(b) Intermittent
- series of short exposures
- one after another
- after various time lapses

(c) Medium
- for a number of days

(d) Chronic
- throughout entire life span (egg to adult death)
- 1 generation - phenocopies?
- somatic mutations?
- changes in physiology and behaviour?

(e) Successive chronic exposure
- continual exposure of 2 or more generations
- gradual deterioration of genetic material? (use reciprocal crosses to determine cytoplasmic and nuclear effects)

(2) Time of treatment withreference to life cycle
(a) From egg stage
(b) At time of highest chemical mutagenic activity (12-48 hours after hatching)
(c) At time of pupation-very specific time and easily recognized
(d) Freshly eclosed adult stage
(e) After aging changes become evident (after 10 days old)

TREATMENT TYPES

(1) Chemical:

(a) Genetic and related effects
- mutagenic chemicals
- various chromosome dyes (possible mutagenic agents)
- nucleic acid analogues
- mitotic inhibitors

(b) Physiological effects
- hydrogen ion concentration of media (may change with time)
- specific metabolic poisons
- osmotic quality of media
- vitamins, hormones and their analogues
- common organic chemicals (eg. urea may affect bonding of proteins)
- common inorganic chemicals (eg. salts of heavy metals)
- antibiotics (should be used only after sterilizing the Drosophila eggs)
- starvation

(2) Physical agents:
  (a) Temperature
    - constant high or low temperature
    -'quantitized' heat or cold shocks
  (b) Space
    - population density during development
    - population density during adulthood
    - densities of different proportions of sexes
  (c) Radiation
    - visible light-different wave lengths (difficult to measure quantitatively)
    - ultraviolet light
    - ionizing radiation-X-rays etc.
  (d) Gas content of environment
    - humidity
    - necessary and other gases

MEASUREMENT OF RESPONSE
  (a) At the population level
    - death rate while under the stress of the chemical or physical agent
    - death rate under normal or other conditions after treatment
    - test chromosomes of the population for mutations
    - rate of egg laying
    - hatchibility of eggs
  (b) Individual Level
    - weight
    -measurement of body components
      count of facet and bristle number
    - determination of symmetry by bristle count on both sides of the insect
  (c) Cellular level
    -affect on mitosis and meiosis
    -examination of giant chromosomes
    -average cell size

STRENGTH OF TREATMENT
(1) As present in natural environment
    - measureable response may take many generations of chronic treatment to be shown
(2) As present only in laboratory conditions

G. SUMMARY

(1) Natural duration of life curves were prepared for Drosophila melanogaster from 10.5 days of age. It was found that the average life expectancy of the males was greater than that of the females.

(2) A mutagenic agent, acridine orange, was introduced into the medium to see if it would modify the aging process. A 10^{-4}M concentration markedly reduced the average life span of D. melanogaster males and females, while a 10^{-5}M concentration reduced the male life expectancy only, having no effect on the females.

(3) A method was developed to test the effect of a chemical stress on D. melanogaster populations. Some preliminary experiments were performed in order to evaluate the effect of 10^{-3}M 2, 4-dinitrophenol, a metabolic inhibitor, combined with various food environments on life duration. Sexual dimorphism in response was found and the relative durations of life of the flies were compatible with a priori considerations. The results suggest a non-linear relationship between the time of 50% population death and the rate of death.

(4) Starvation death curves were compiled for D. melanogaster following exposure to various conditions which were thought to be representative of the natural environment. Under starvation conditions female Drosophila were found to outlive their male counterparts in all these experiments. The female life duration markedly increased after the population had been kept under optimum conditions thus making a greater difference between these starvation life expectancies of males and females as compared to the less optimum environments.

(5) Utilizing a combination of a rapid 'quantitized' heat-shock and starvation conditions it was found that the starvation life duration of the males was significantly decreased compared with the unshocked starved controls. The female starvation life duration did not appear significantly decreased due to the heat-shock. Under starvation conditions wholly female populations had a life duration similar to populations comprised of equal numbers of both sexes.

(6) Recommendations for further experimentation are made.

H. APPENDIX

Medium No. 1
- 600cc water
- 7.5gms agar
- 50cc molasses
- 7.5gms debittered yeast
- 50gms corn meal
- and a few drops live yeast suspension

The water is brought to boiling and the agar is added and dissolved. The other ingredients are then mixed in.

Medium No. 2 - Medium No. 1 with live yeast omitted

Medium No. 3 - no live yeast, no cornmeal or debittered yeast (otherwise Medium No. 1)

Medium No. 4
- 2cc 10^{-3}M acridine orange
- 18cc of Media No. 1 using only the water, agar, and molasses in the same proportion (Media No. 3)

Medium No. 5
- 2cc 10^{-4}M acridine orange
- plus 18cc Media No. 3
- Normal - 20cc Media No. 3

Medium No. 6
- 10cc of 15X 10^{-4} M 2, 4-Dinitrophenol
- 3cc of 5% fructose
- 2cc salt solution[1]
- plus debittered yeast on wick

Medium No. 7 - Medium No. 6 with debittered yeast omitted

Medium No. 8
- 10cc of 15 X 10^{-4}M DNP
- 3cc of 20% fructose
- 2cc salt solution

[1] the salt solution consisted of

- $NaHCO_3$	0.700
- KH_2PO_4	0.914
- Na_2HPO_4	0.945
	% by wt.

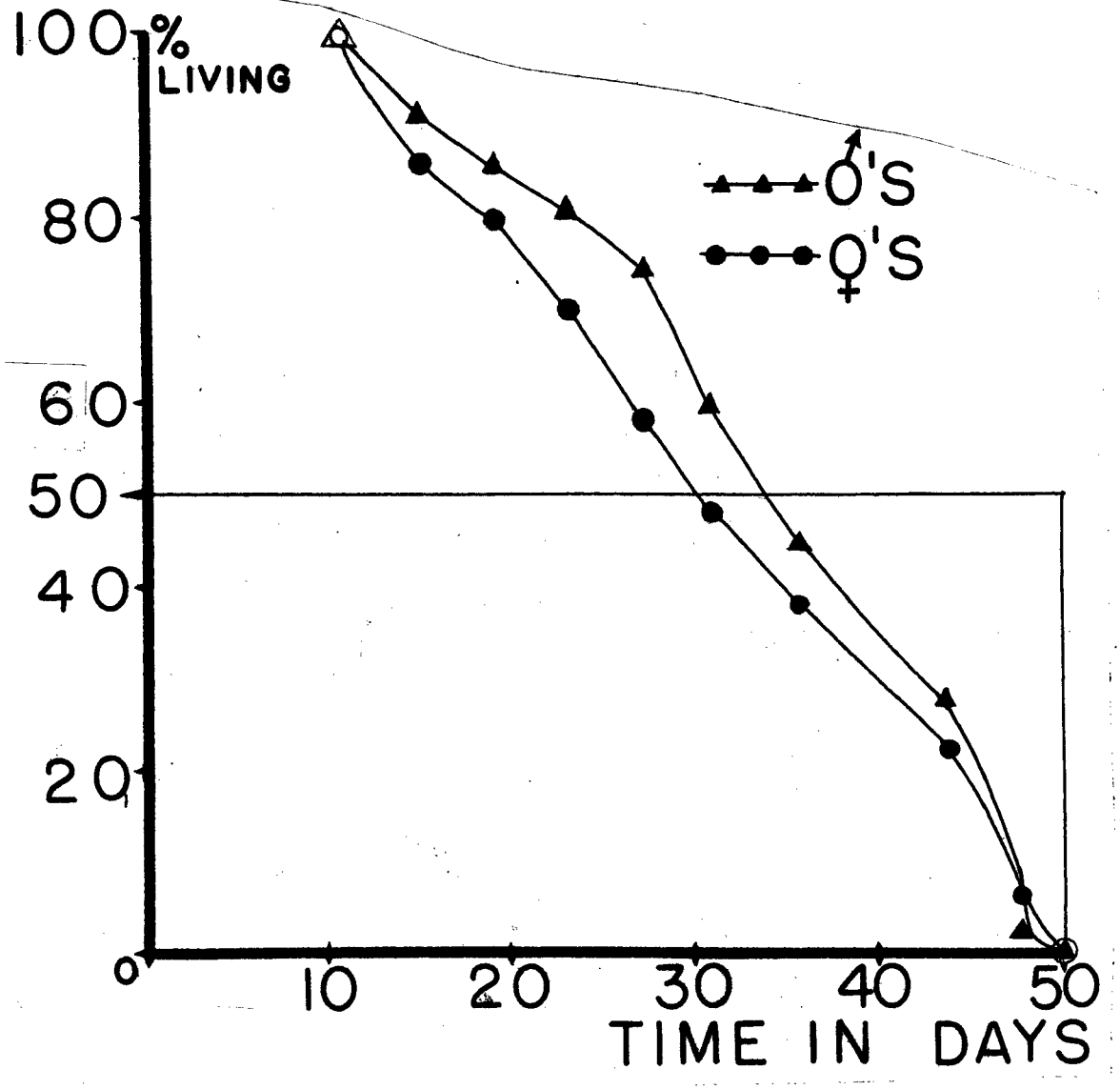

Figure 1a
Natural death curves of Drosophila melanogaster males and females

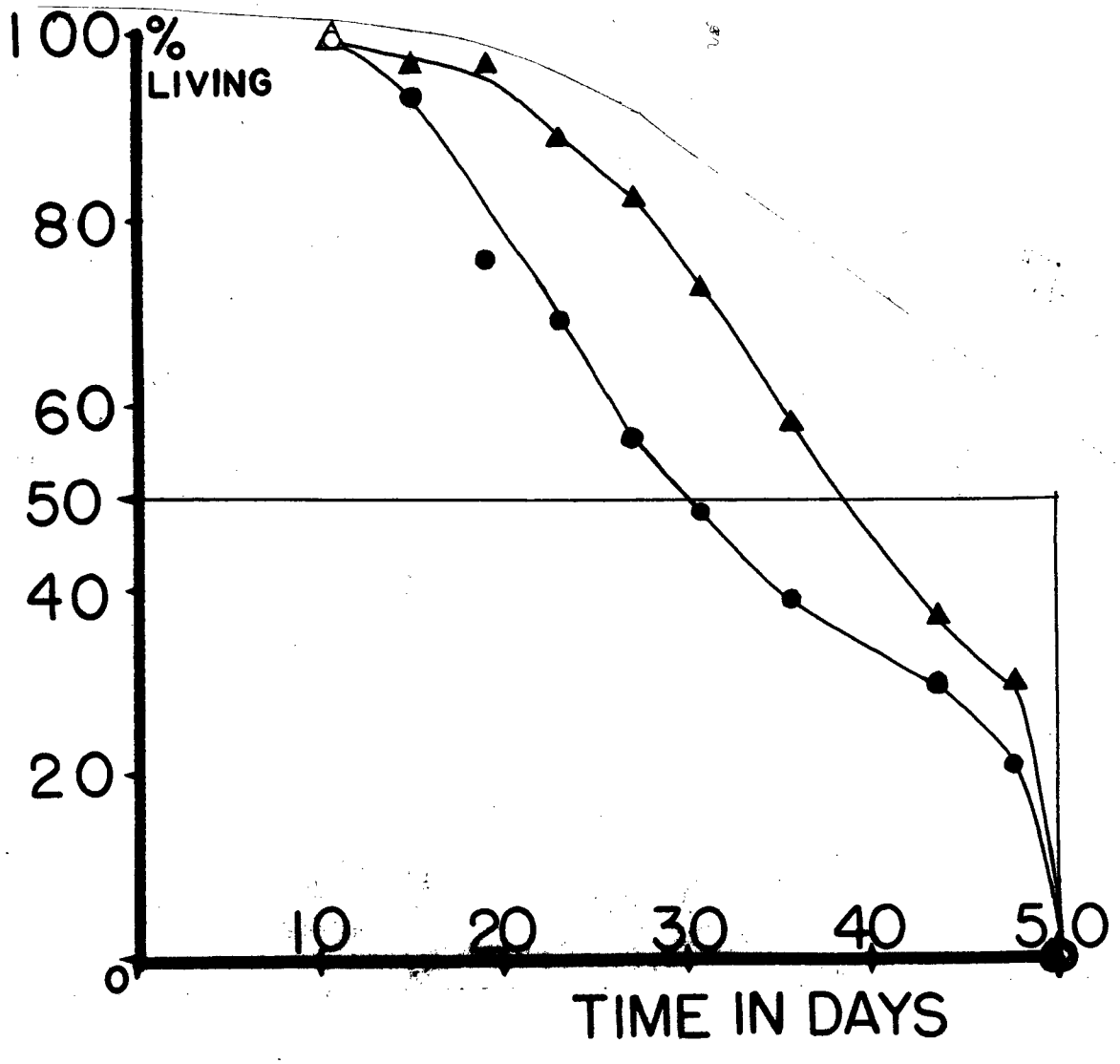

Figure 1b
Natural death curves of Drosophila melanogaster males and females

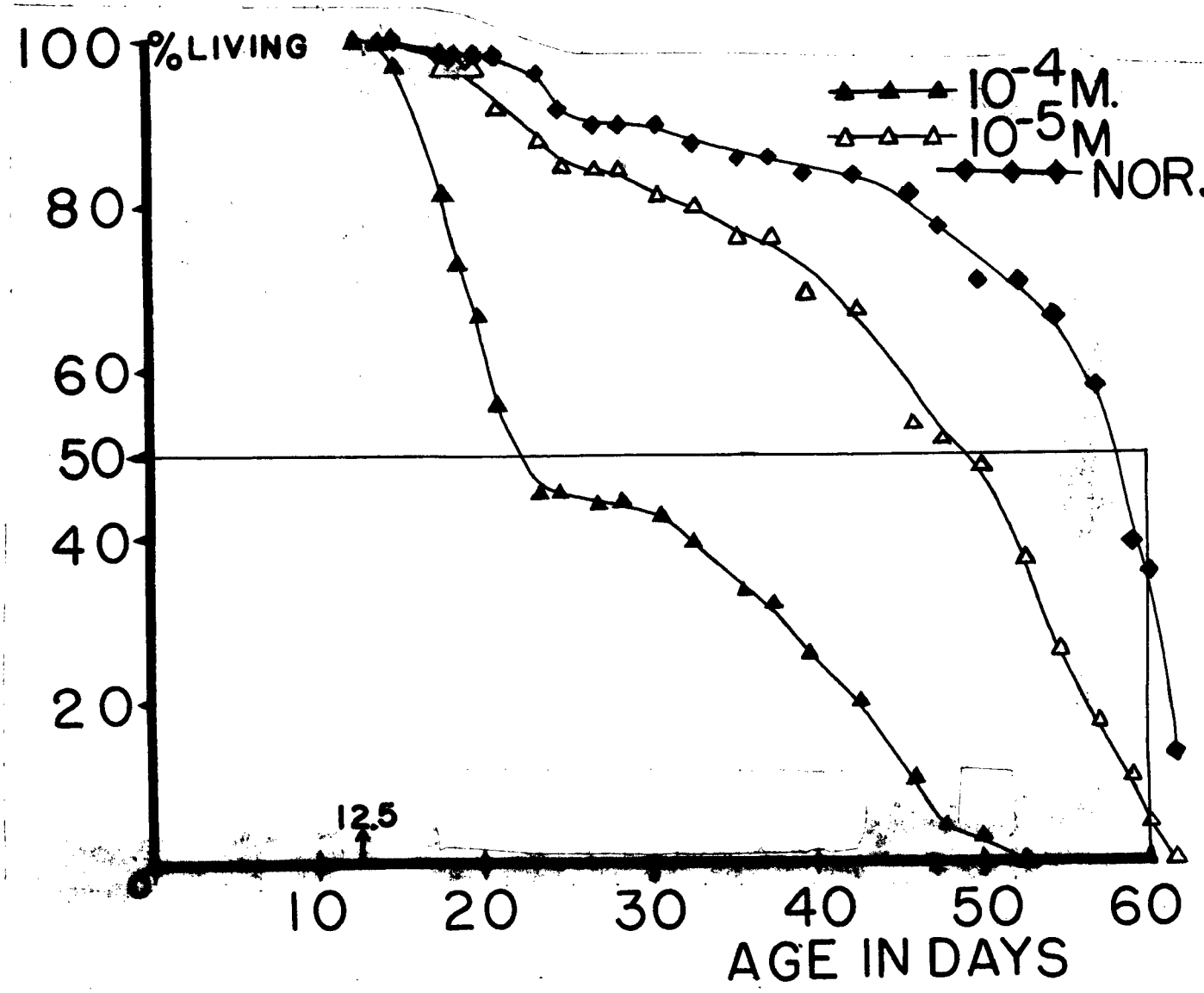

Figure 2a
The effect of various concentrations of acridine orange on the life span of Drosophila melanogaster males

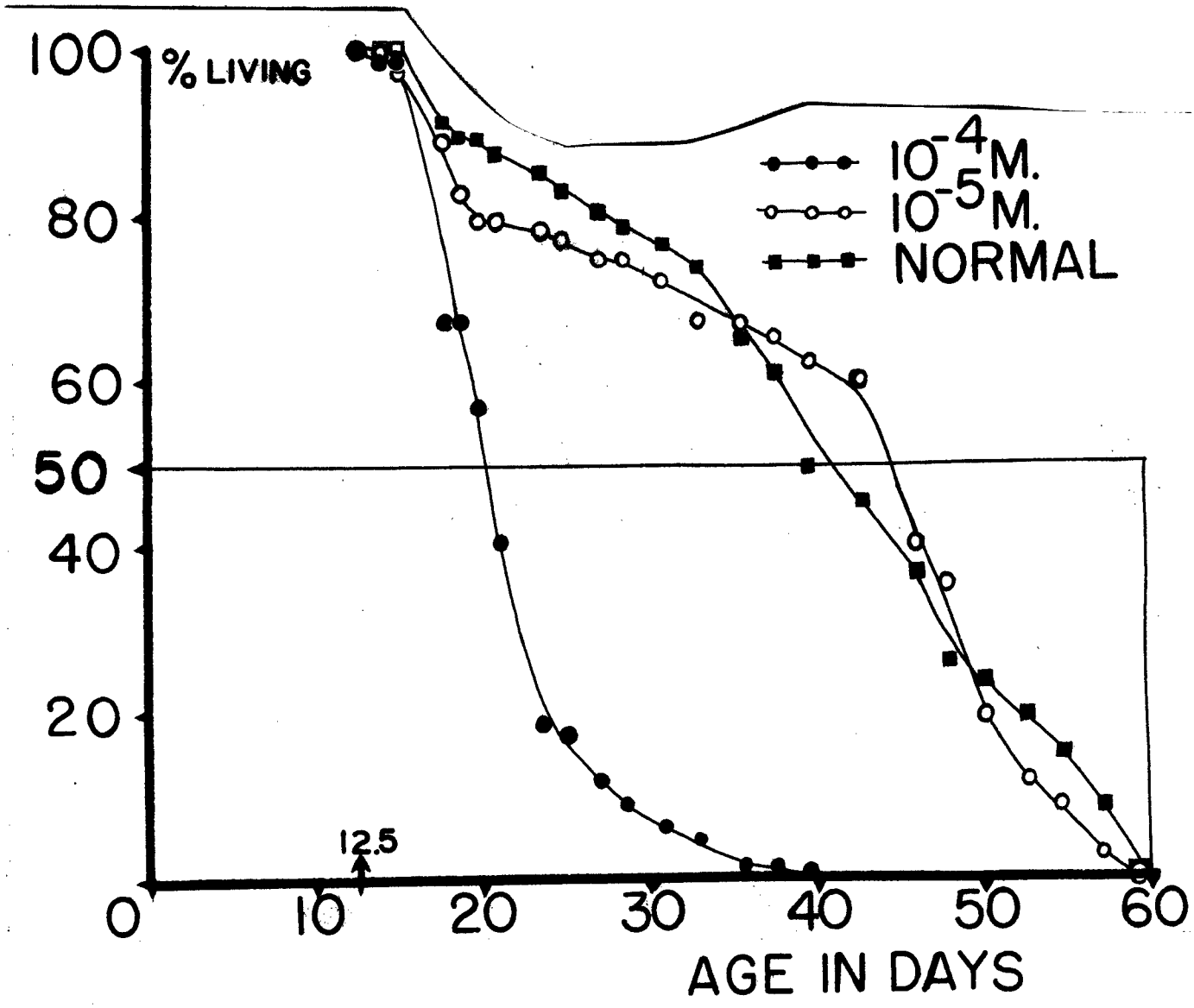

Figure 2b
The effect of various concentrations of acridine orange on the life span of Drosophila melanogaster females

POPULATION CONTAINER

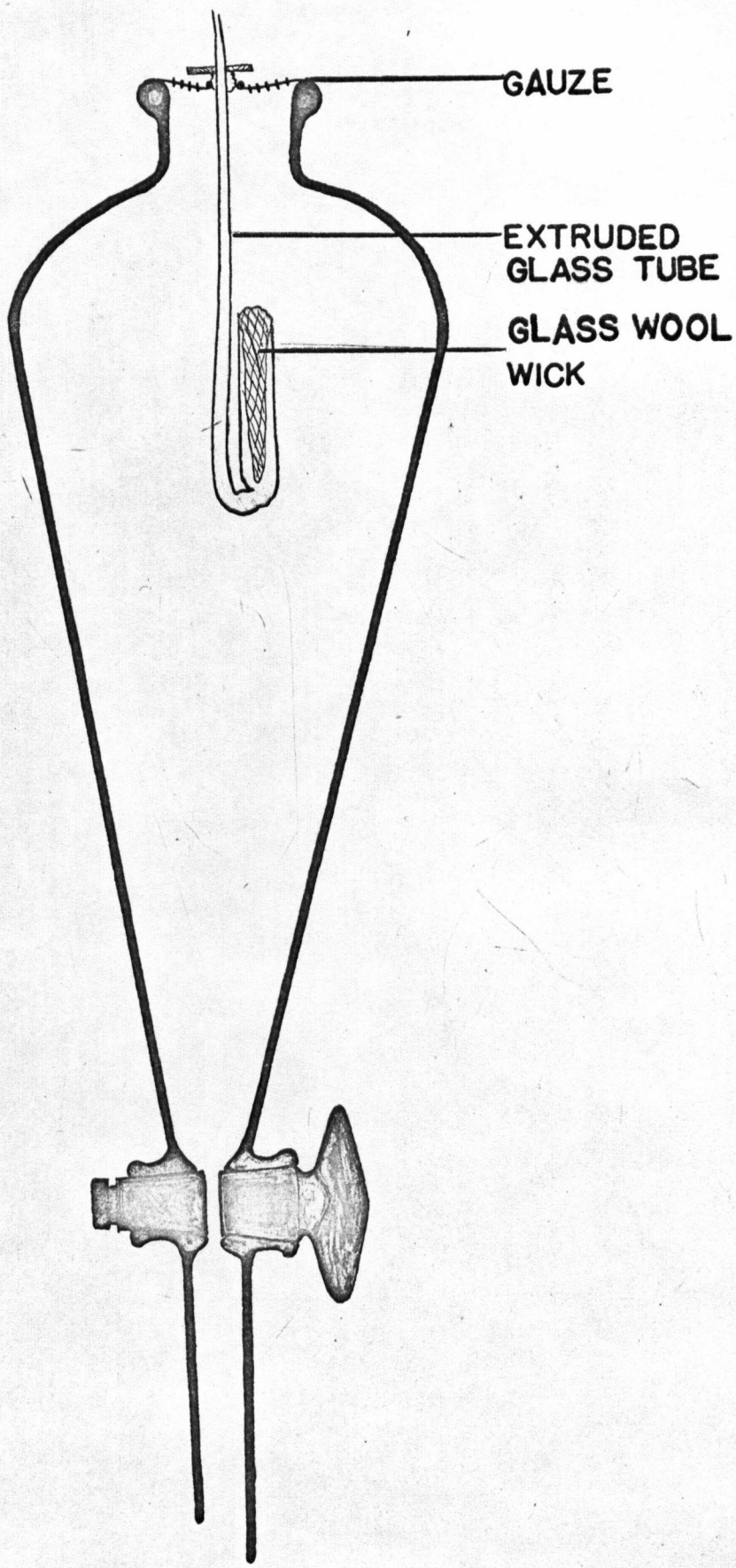

Figure 3a
Population container

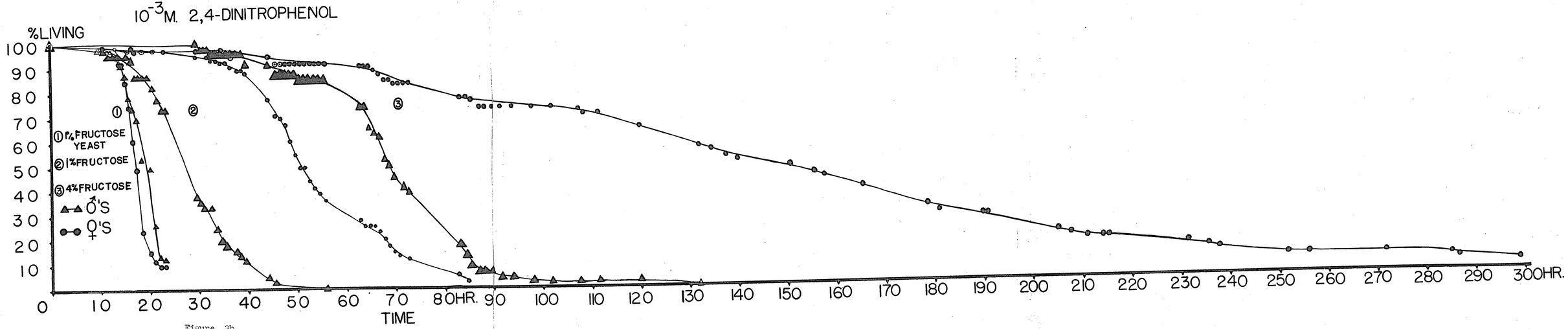

Figure 3b
Death curves of Drosophila melanogaster males and females exposed to DNP and various food environments

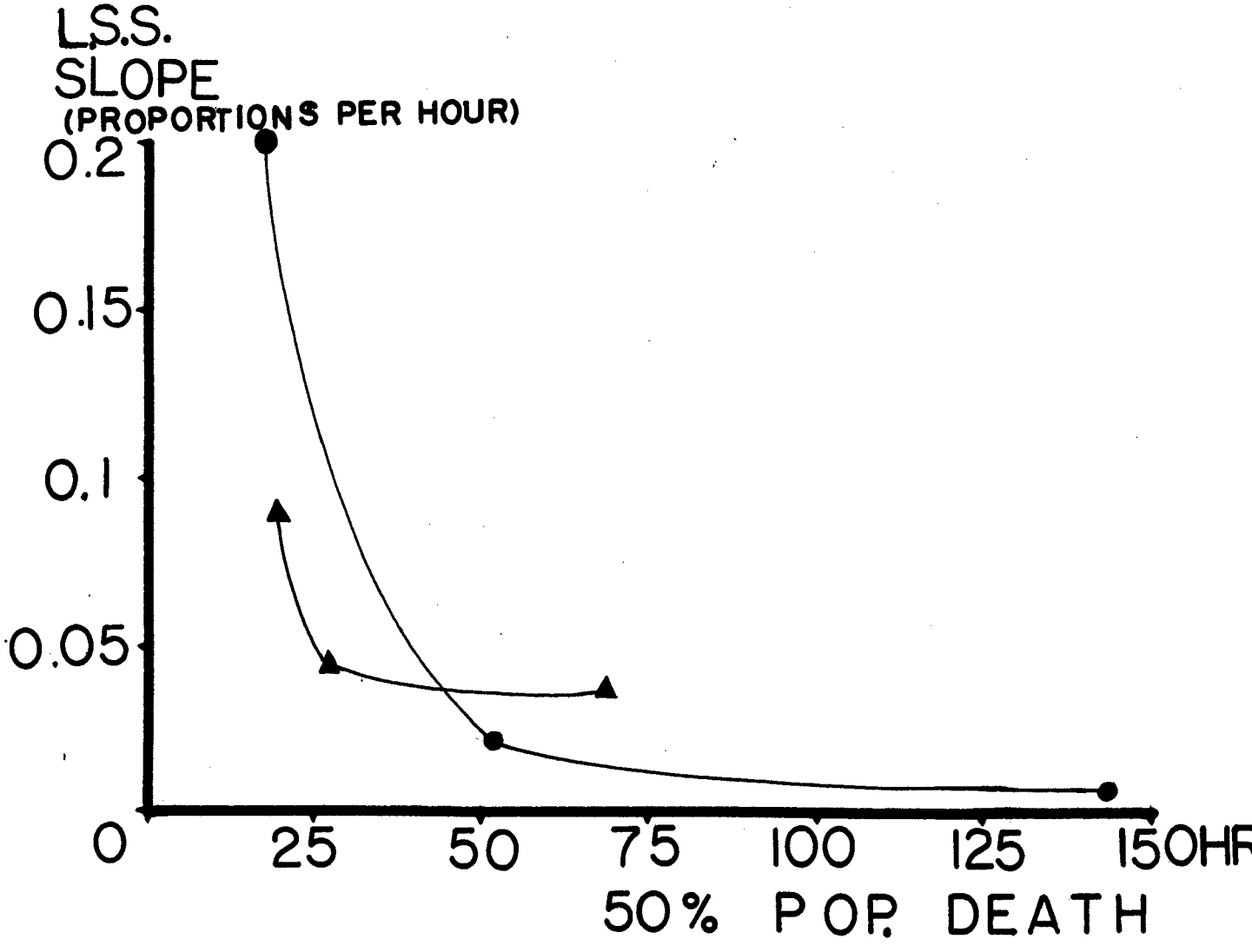

Figure 3c
Least Squares Slope between 20 and 80% versus the 50% population death time for the DNP experiments

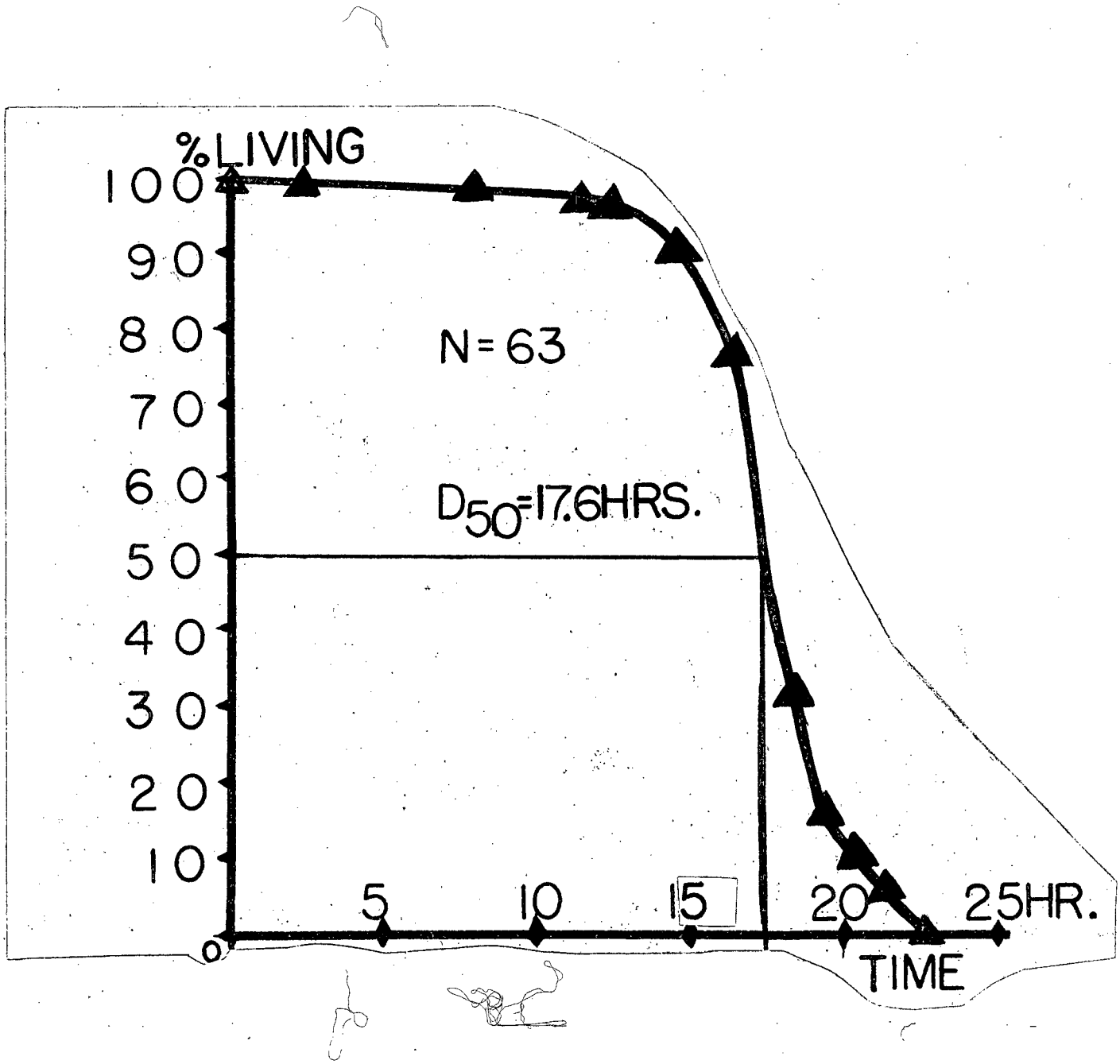

Figure 4a
Starvation death curve of Drosophila melanogaster males

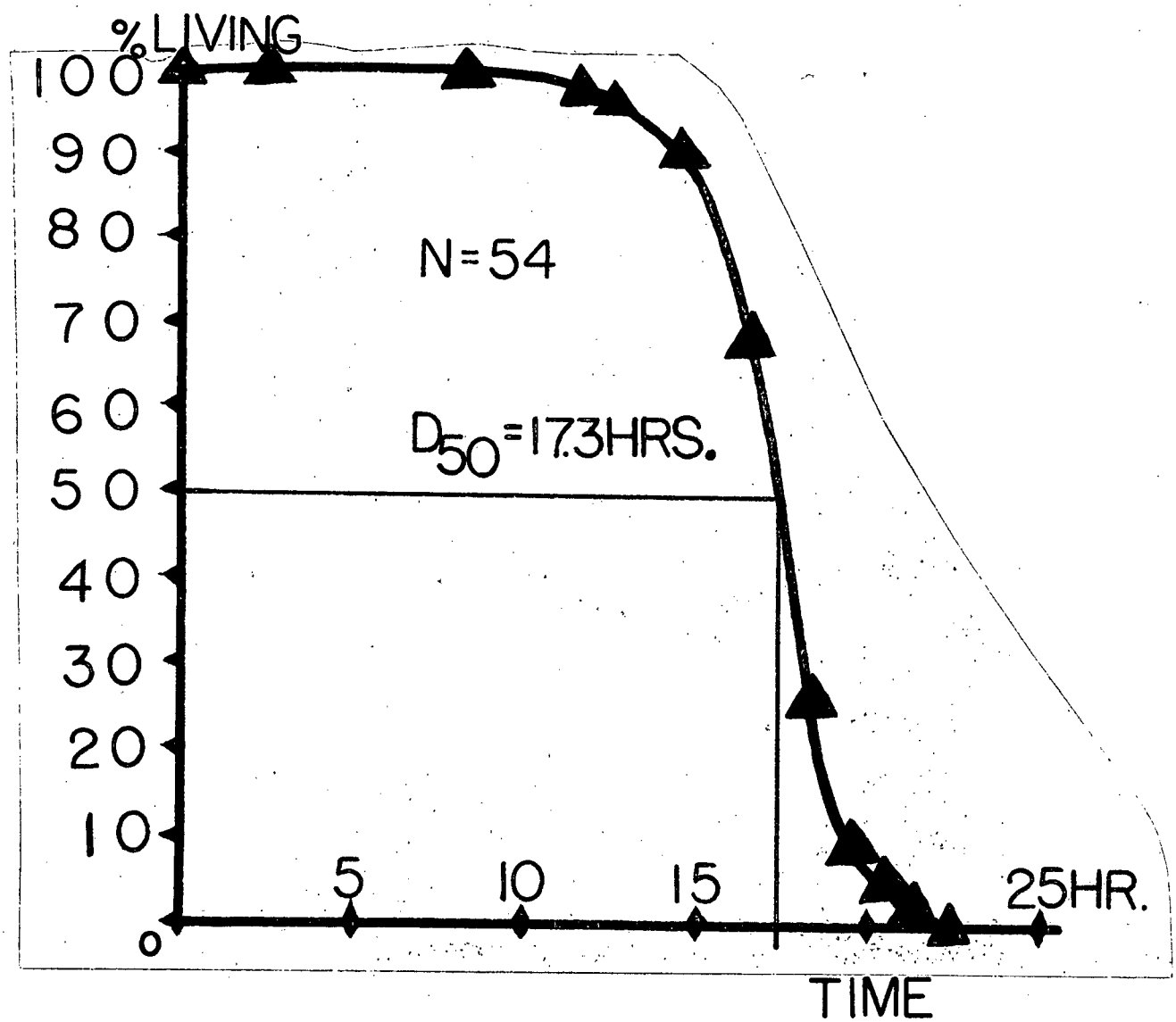

Figure 4b
Starvation death curve of Drosophila melanogaster males

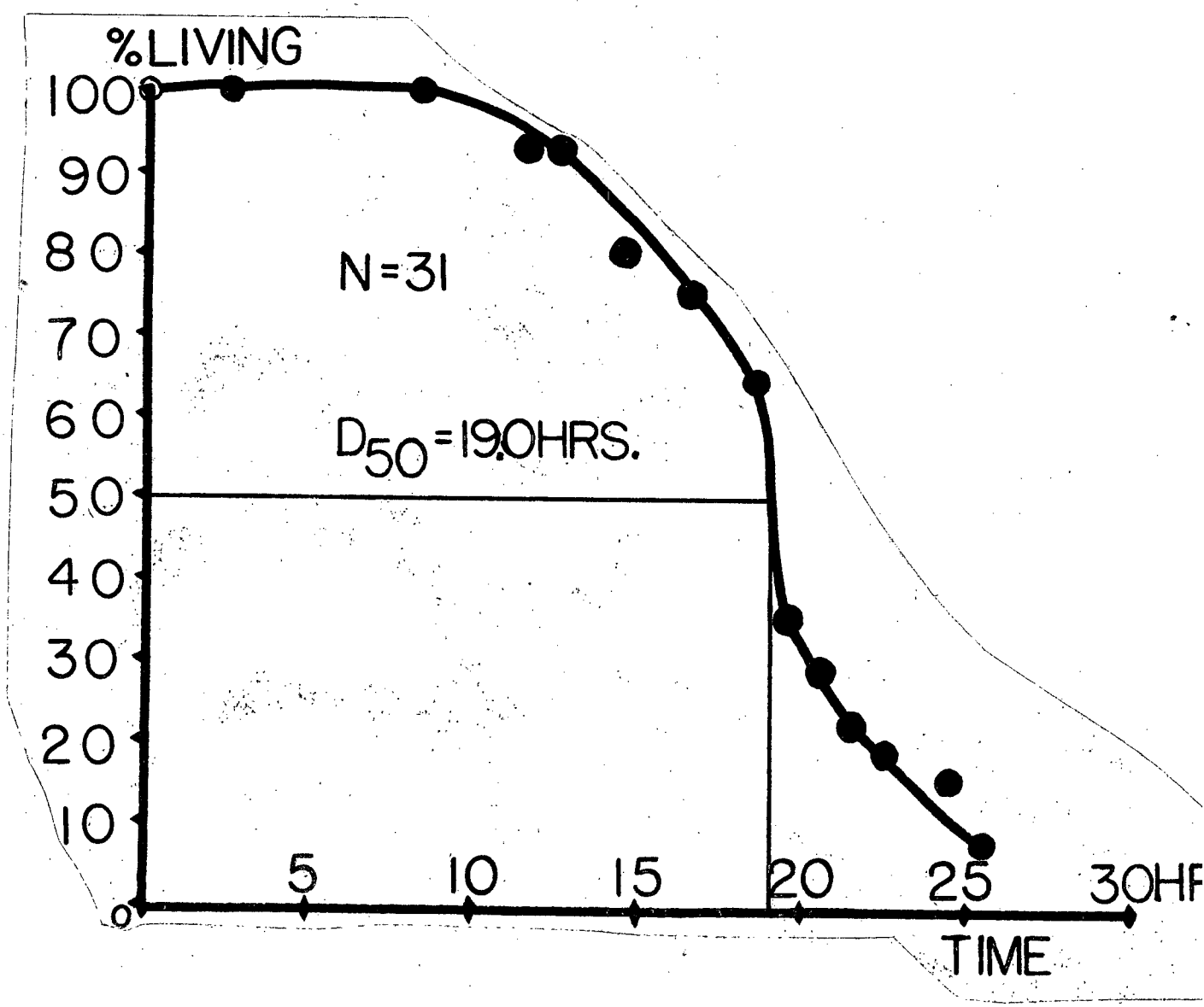

Figure 4c
Starvation death curve of Drosophila melanogaster females

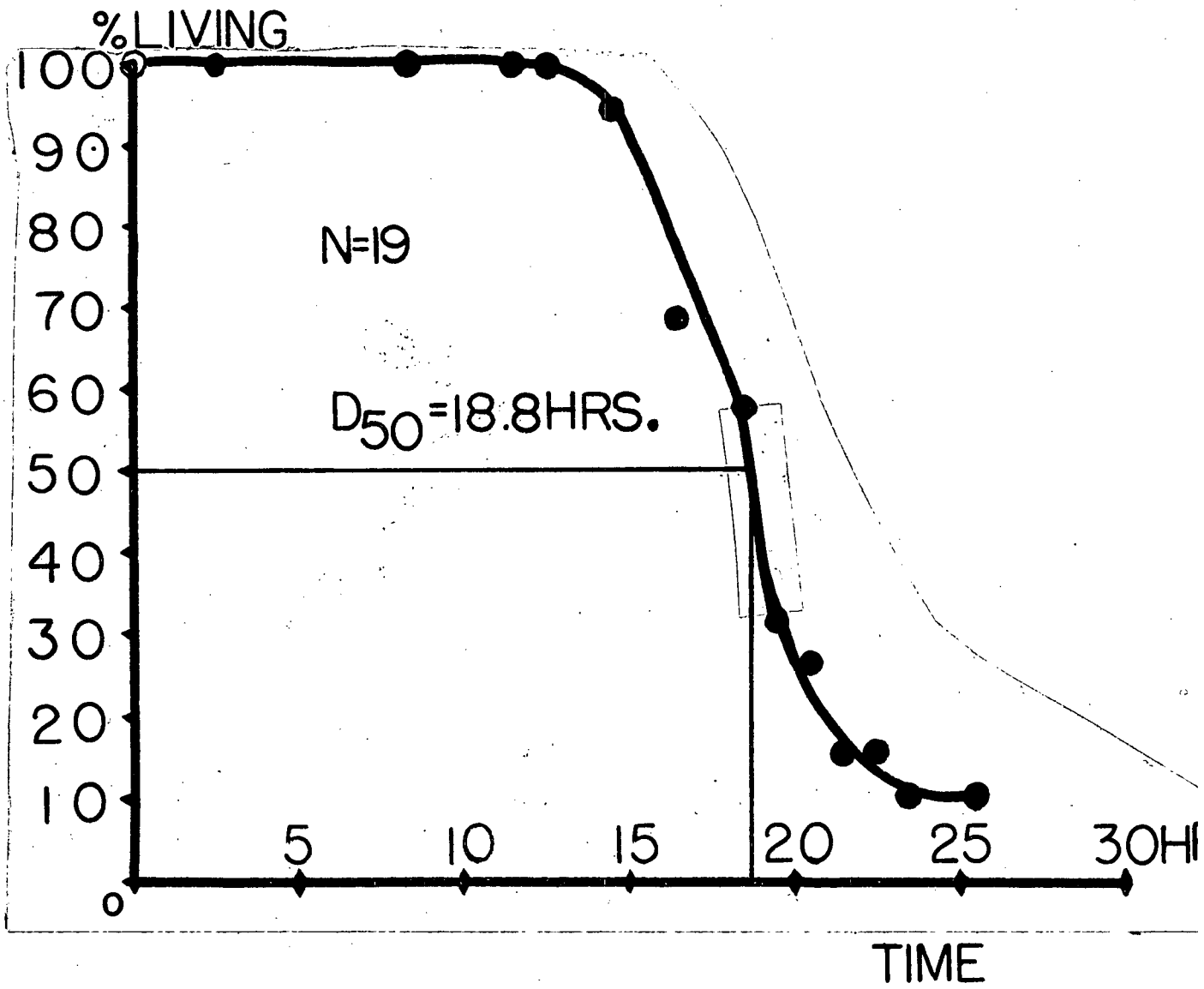

Figure 4d
Starvation death curve of Drosophila melanogaster females

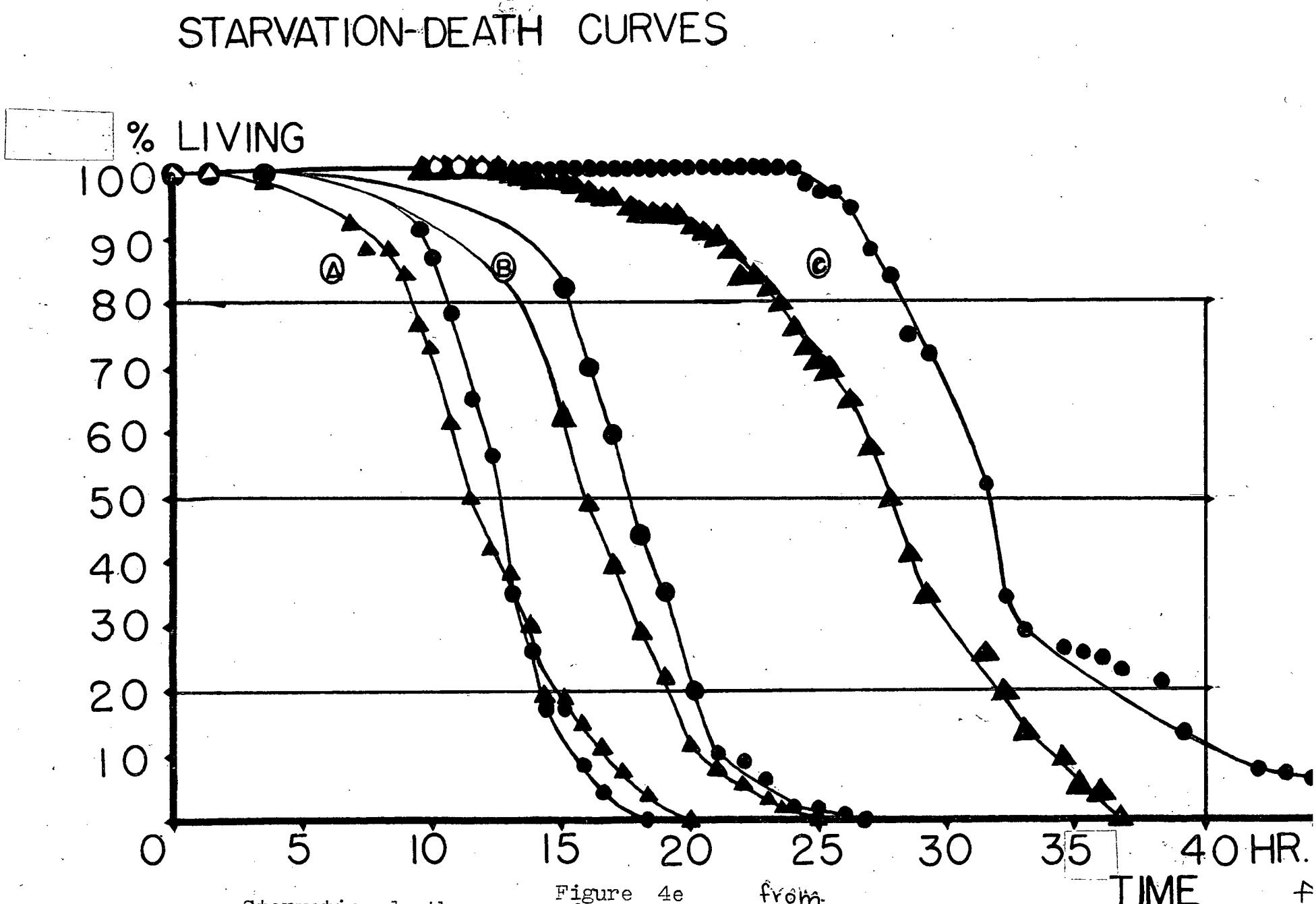

Figure 4e from

Starvation death curves of Drosophila m. ~~in~~ various environments

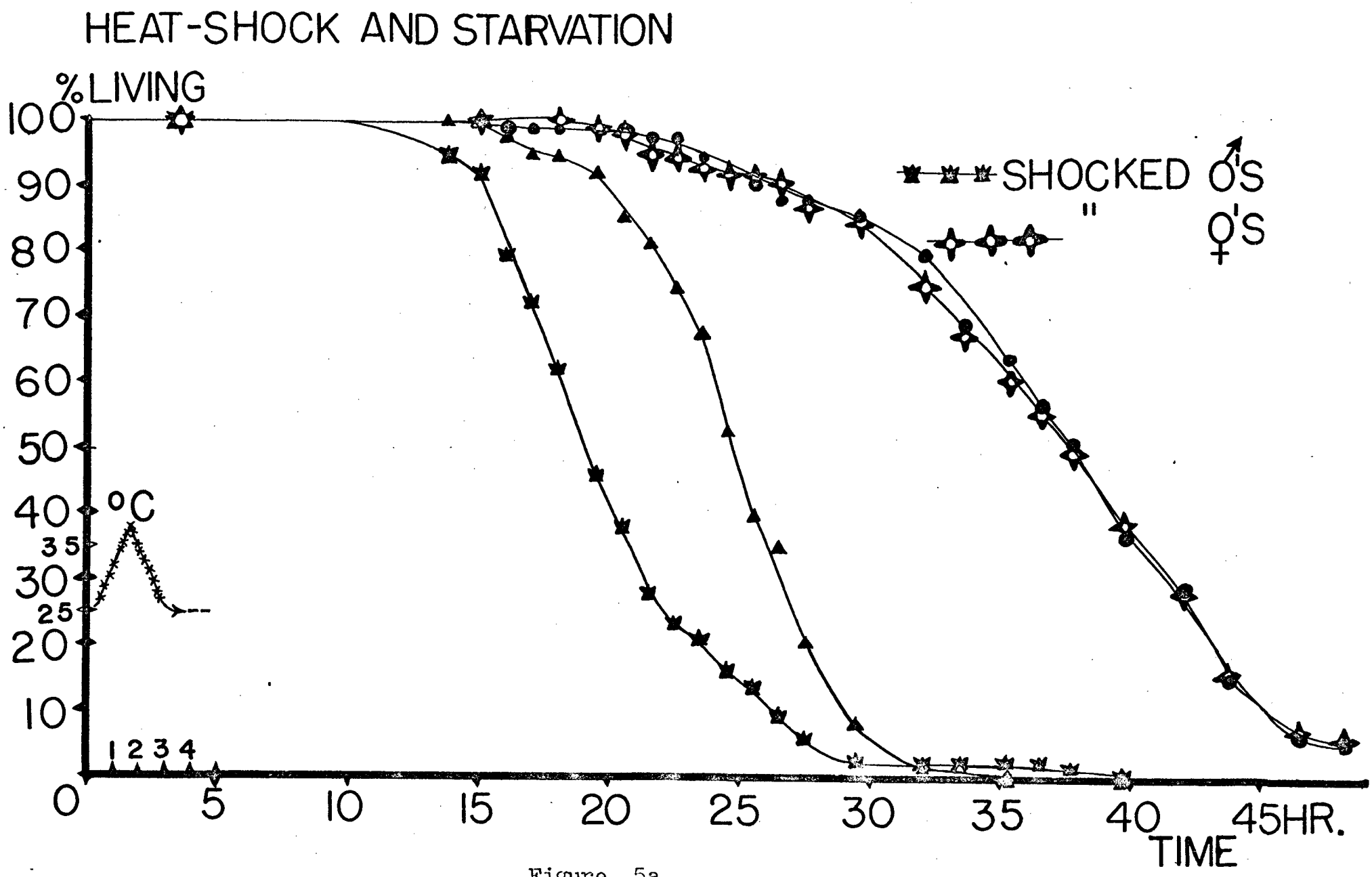

Figure 5a
The effect of heat-shock and starvation on Drosophila m. males and females

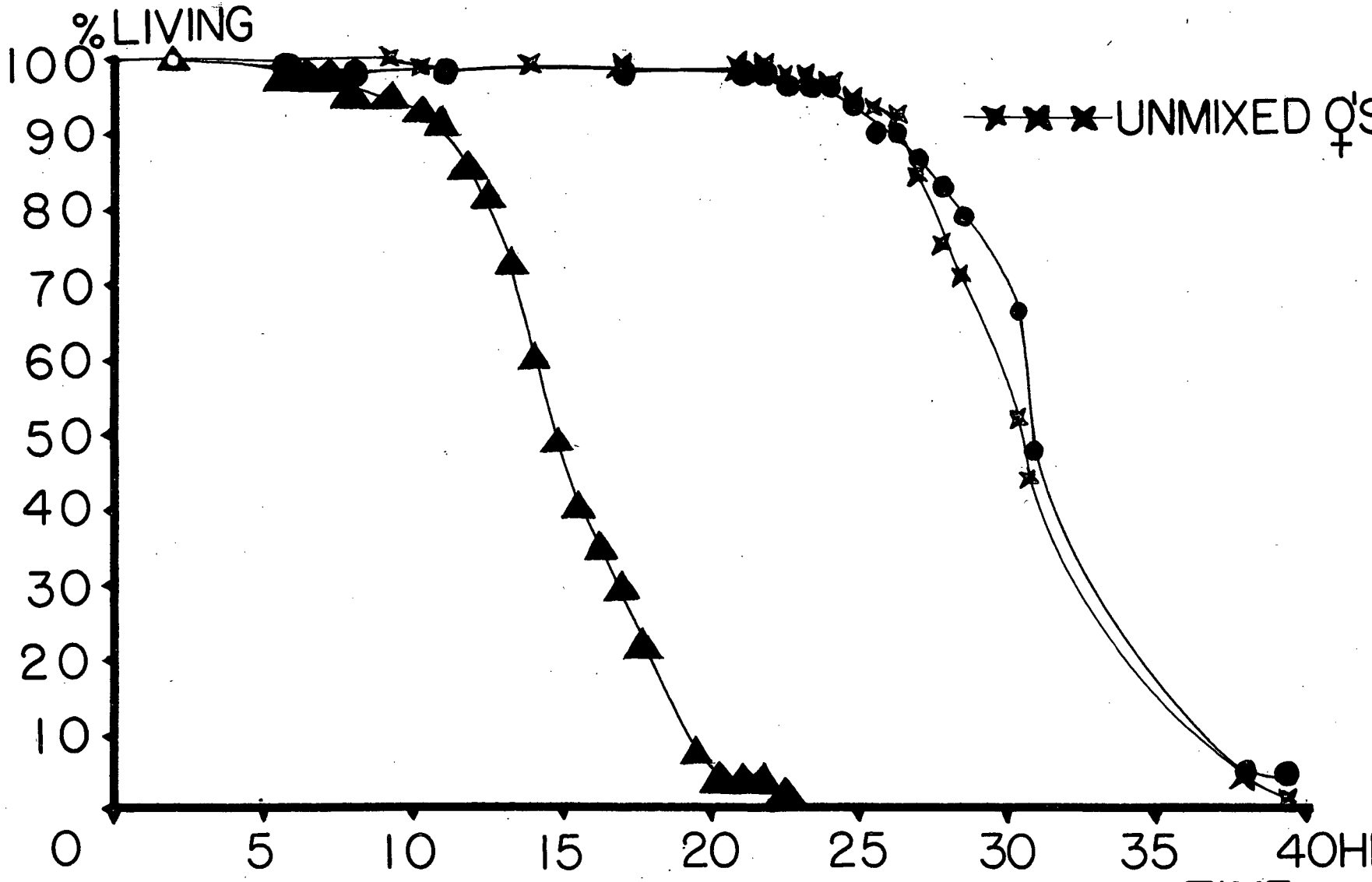

Figure 5b
The effect of starvation on mixed and unmixed Drosophila melanogaster females

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