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"The effects of sex and nutrition on aging of the free-living diecious nematode *Panagrellus redivivus* (N strain) in axenic culture"

by

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"The effects of sex and nutrition on aging of the free-living diecious nematode Panagrellus redivivus (N strain) in axenic culture"

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Abstract

Under standard axenic conditions at room temperature, males of the free-living nematode *Panagrellus redivivus* (N strain) have a mean life span of 29.26 days while females live for an average of 36.29 days. A delay in the completion of the post-embryonic development by nutritional means, results in an increase in the mean life expectancy. Reduction of the developmental period by increasing temperature to 30°C, results in a decrease in the mean life span of males and females to 20.90 and 24.46 days respectively. That these are a measure of senescence, is shown by the sex specific pattern of increased osmotic fragility and decreased behavioural activity as a function of time.

It appears that in the normal course of post-embryonic development of *P. redivivus*, the onset of aging is triggered by a specific developmental event. The timing of this trigger seems to coincide with the completion of the final moult.

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Introduction

Any biological system has its own characteristic life span. During this period, it first undergoes the process of development expressed as a complex series of changes in its biological organization and activities. As a function of time, the level of these activities and/or biological organization begins to decline and the organism undergoes the process of senescence (aging) which results in death. Aging is a consequence of differentiation and therefore a product of the genome and the environmental conditions of a given organism.

The inherent complexity and long life span of higher multicellular organisms makes them unsuitable systems for obtaining a precise analytical resolution of the total processes of aging. Attention has therefore turned to distinct organs and cells in tissue culture. The high and varying cell turn over rate of organs make them unsuitable. It is difficult to ascertain whether cells in culture are normal and would provide the precise information on the nature of aging as it occurs in vivo. Studies on single cellular products as a function of age are too simplistic and ignore the totality of the syndrome of senescence.

More recently, attention has focused on simpler lower multicellular organisms as model systems for aging studies. One such a system is the nematode.

Nematodes are suitable systems because some of them have a very short generation time and rapid reproduction rate. The post embryonic period comprises four juvenile stages and a sexually mature adult stage. Each stage is separated from the next by a moult which occurs at specific intervals in the life cycle. Cell division ceases prior to the first larval stage in all tissues except the reproductive system. Along with these features, several free-living and parasitic forms may be grown in axenic culture. The simple structure of nematodes allows simple microscopic examination.

In addition, nematodes along with some Aschelminthes like Rotifers and a few lower metazoans, e.g., Coelentrates, Arthropods and Molluscs have the characteristic ability to slow down (quicescence) or even shut down their metabolism (cryptobiosis) with a shift to unfavourable conditions (Cooper and Van Gundy, 1971). Thus, structural integrity could be maintained or only slowly decline and a potential for resumption of motility or even normal senescence is retained. This could provide at least in theory a system for modifying and delaying aging through the control of environmental conditions.

Cooper and Van Gundy (1971) reviewed some of the earlier studies of aging in parasitic nematode species. Those studies were basically restricted to specific larval stages and the results were complicated by starvation and other exogenous effects.

Recently, attention has focused on the use of free-living nematodes for the study of aging (Gershon, 1970; Erlanger and Gershon, 1970; Gershon and Gershon, 1970; Zuckerman, Himmelhoch, Nelson, Epstein, and Kisiel, 1971; Zuckerman, Nelson and Kisiel, 1972; Kisiel, Nelson and Zuckerman, 1972; Reitz and Sanadi, 1972; Zuckerman, Himmelhoch and Kisiel, 1972; Kisiel, Himmelhoch and Zuckerman, 1973; Beguet and Brun, 1972; and Beguet, 1972).

Gershon, (1970); reported that *Turbatrix aceti* ages with 50% survival at 25 Days and that DNA synthesis is not required for the maintainance of normal life span and growth. The activity of the enzymes - acetylcholinestrase, α - amylase and malic dehydrogenase decreased with age in *T. aceti* while that of acid hydrolase increased for the first 15 days and remained at an elevated level up to day 25 after which considerable decrease in activity occurred (Erlanger and Gershon, 1970). Gershon and Gershon, (1970) used immunological techniques to compare young and old *T. aceti* and found enzymatic differences. Reitz and Sanadi,

(1972); used hydroxyurea treated *T. aceti* and found differences in iso-accepting tRNA in old nematodes and concluded that it represents an index of possible changes at the translational level of protein synthesis.

Zuckerman, et. al., (1971); reported that the hermaphroditic nematode *C. briggsae* has an average mean life expectancy of 28-30 days at 25°C and that increase in temperature to 27°C results in the shortening of the mean life span and generation time. Other changes associated with aging in *C. briggsae*, were reduced activity and decreased ability to withstand osmotic stress. This latter change was correlated to increased specific gravity with age (Zuckerman, et. al., 1972). Ultra-structural studies on aging *C. briggsae* revealed increased degeneration of hypodermal mitochondria and formation of lysosome-like bodies in the interchordal hypodermis (Zuckerman, et. al., 1971). Fine structural studies on the cuticle of old *C. briggsae* showed that the outer osmophilic layer became more defined while electron-dense material and electron-dense balls accumulated within the fluid filled layer (Zuckerman, et. al., 1973).

Beguet and Brun, (1972); found that parental aging in *Caenorhabditis elegans* grown monoxenically results in reduction of the number of F_2 off-spring

produced by F_1 parents. Beguet, (1972); maintained young (5 days old) and old (8 days old) *C. elegans* parental series over nine consecutive generations and found that the average fecundity of the old series reduced over the first four generations and then returned to the same level as that of the young parents after the 5th generation. He suggested that this could be linked to the initial disorganization of homeostatic processes of old parents.

Normally, the aging syndrome begins to appear after the completion of the reproductive phase of the life cycle. Nematode aging studies have concerned themselves with the reproductive processes. With *C. briggsae*, reproduction was allowed to occur by growing the nematodes in isolation before assaying for aging (Zuckerman, et. al., 1971). It is impossible to obtain large populations of nematodes of known age at a given time for biochemical studies without inhibiting reproduction.

The reported effects of DNA synthesis inhibitors on nematodes has been contradictory. Zuckerman, et. al., (1972); cite Diekx as having found that high concentrations of acridines inhibited growth and caused death before adult stage in *Panagrellus silusiae*. Low concentrations

of acridine delayed maturation and the few animals that matured, did not reproduce. Sayre, Hansen and Yarwood, (1963); reported that anethopterin plus leukoviron permitted full growth but not reproduction of *C. briggsae*. Pasternak and Samoiloff, (1970); reported that nalidixic acid, phenethyly-alcohol and hydroxyurea has slight growth inhibitory effects on *P. silusiae*. Gershon, (1970); found near normal growth and maturation of *T. aceti* treated with aminopterin, fluorodeoxyuridine and hydroxyurea at dosages which prevented reproduction. In the animal parasitic nematode *Nippostrongylus brasiliensis*, Bollar, Bonner and Weinstein, (1972); found that moult from the second stage to third stage larva is inhibited by actinomycin D and the nematodes are smaller than normal. Kisiel, et. al., (1972); reported that concentrations of hydroxyurea, aminopterin and 5-fluorodeoxyuridine which prevented reproduction, caused significant growth inhibition, prevented maturation past the third larval stage and reduced longevity of *C. briggsae* and *T. aceti*. Further, most of the treated nematodes had cuticular blisters while low dosages of aminopterin sometimes prevented vagina formation and caused hatching of the eggs within the oviduct. Aminopterin treated *C. briggsae* are unable to adjust to the osmotic pressures tolerated by untreated nematodes of the same age. Inhibition of DNA synthesis according to

Boroditsky and Samoiloff, (1973); blocked morphological organization of the gonad despite normal cell proliferation but had little effect on growth of *Panagrellus redivivus*. Westgarth-Taylor and Pasternak, (1973); reported that in the diecious nematode *Panagrellus silusiae*, long term treatment with hydroxyurea caused cuticular abnormalities.

It is obvious that drug-treated nematodes are abnormal in several respects. One must question the use of such drug-treated populations for aging studies. It has been questioned whether any observed changes are in fact age related and not primarily associated with exposure to chemicals (Kisiel, et. al., 1972). Because nematodes are mainly eutelic, the major developmental events are primarily in the reproductive system. Thus, inhibition of the development of the reproductive system might not provide the true information in aging assays.

Cryan, Hansen, Martin and Yarwood, (1963); found that in axenic cultures of *P. redivivus*, 60-80% of the living adults in the terminal populations were females and there was a predominance of males among the dead. Culturing the free-living nematode *Panagrellus silusiae* on infants food Gysels, (1965) found that at an average of 30°C, the male/female proportion increases with increasing number of nematodes. He suggested that

femalessecrete male inhibiting substances which cause the early death of males. Hansen and Cryan, (1966) also found that the sex ratio of *P. redivivus* varies in response to heat and nutritional stress with a high predominance of mature males and lack of maturation of females. These suggest sex differences which may be due to differences in aging rate.

One important factor which has been ignored in aging studies of nematodes is the role of developmental rate on longevity and how much it affects the timing of the appearance of age related characteristics. The extension of developmental period by under-feeding has been suggested as one of the possible means of extending the mean life span of animals, (Strehler and Burrows, 1970). Metabolic retardation (calorie restriction) by under-feeding however, requires tentative identification of its net effects particularly in terms of morphology and physiology. Nematodes in axenic culture could serve as good experimental tools in this direction. This is because they adapt to environmental control mechanisms without showing appreciable disabilities and can recover when such stress are removed.

In this investigation, aging in the free-living diecious nematode *Panagrellus redivivus* (N strain) in

axenic culture was assayed by examining age related response to hypotonic shock, behavioural changes and the effects of various nutritional conditons on survival. The effect of temperature increase on longevity was also investigated. The possible role of the various components of the culture medium in growth, gonad development and general morphology as well as the extent to which one of these nutritional components affects survival is also reported.

Materials and Methods

Nematode Culture

The original culture of the free-living diecious nematode *P. redivivus* (N strain) used in this study was obtained from Dr. E. L. Hansen, Institute of Clinical Pharmacology, Berkeley, California, and has been maintained in medium consisting of 4% soy peptone and 3% yeast extract (SPYE) as the basal medium and 10% heated liver extract (HLE) as the supplement.

Mass cultures were maintained in 5 ml medium in 120 ml flat bottomed culture flasks, while smaller cultures were grown in 1-2 ml medium in 10 ml screw capped vials. All cultures were incubated at room temperature (approximately 25°C).

Glassware was autoclaved for 20 minutes at 18 lbs pressure and 121°C. Innoculations were carried out in a transfer box equiped with a 15 w General Electric "Germicidal" ultra-violet light running the length of the box just beneath the top. The germicidal light was left on except when culture transfers were being made. A continuous stream of sterile air filtered through 0.2 μ pore size gas filter was continuously maintained through the box.

No standard sterility checks were carried out as the culture medium serves as its own check by turning milky within 24-48 hours of innoculation if there is bacterial growth.

The culture medium

SPYE was prepared by dissolving 4 grams of soy-peptone and 3 grams of yeast extract (Oxoid Ltd., London No. L21) in 90 mls of distilled water, (Sayre, Hansen and Yarwood, 1963). Preparations were autoclaved for 15 minutes at 15 lbs pressure and 121°C. Autoclaved SPYE was stored in the refrigerator at 5-10°C until used.

The organic supplement HLE, was prepared according to the method of Sayre, et. al., 1963. This was done by first grinding commercial beef or calf liver in a meat grinder after excising it of its connective tissue. The ground liver was then weighed and homogenized with an equal amount of distilled water (w/v) for 5 minutes in a Waring blendor. The homogenate was then heated to 55°C in a water bath and maintained at that temperature for 10 minutes with continuous stirring. The heated coagulated homogenate was then cooled to 10°C in an ice-bath with continuous stirring. The cooled coagulated homogenate was then passed through 2-3 layers of cheese cloth to remove as much of the coagulum as possible and

centrifuged for 30 minutes at 17 x 10^3 r.p.m. in a Sorval RC-2 refrigerated centrifuge (0°C). The supernatant was decanded and prefiltered through a series of pre-filters of pore size ranging from 10 M to 0.4 M. Final sterility was achieved by passing the filtrate through a membrane filter of pore size 0.2 M. The sterile HLE can be stored frozen for 2-3 months without any appreciable fall in activity (Sayre, et. al., 1963).

Due to initial difficulties in obtaining sterile HLE, cultures were maintained in SPYE supplemented with either 10% autoclaved liver extract (ALE) or ALE + Casein (0.03 gms/ml) (Rothstein and Cook, 1965). These media required constant re-innoculation and the practice was discontinued when sterile HLE could be more easily obtained.

(A) Population growth

Culturesfor population growth studies were maintained in 120 ml glass culture vessels with thin walls to permit easy microscopic examination of the nematodes. Synchronous populations of second stage larvae were obtained by passing a large culture of mixed stages over a 5-6 cm column of sterile glass micro-beads

(0.3 mm diameter), (Samoiloff and Pasternak, 1968). Under such conditions the first 10-30 drops consists mainly of second stage larvae (350 ± 7M in length). One ml of the medium together with the suspended L_2 was then introduced into 4 mls of fresh culture medium (SPYE + HLE). This forms a thin layer (1 mm thick). At two days intervals, 3 television recordings of the culture were made with the culture shaken between readings to ensure even distribution of the nematodes. Using 1mm square graph sheets, it was possible to obtain the area of the culture projected on the television screen at any given time. At the end of the experimental period, the number of nematodes on the screen at each time of recording was obtained. The experiment was repeated three times.

(B) Assay for aging

a) Response to hypotonic shock.

The response of 4, 8, 16 and 24 days old nematodes which had been grown individually (virgins) in 0.2 ml SPYE + HLE in ¼ drum screw capped vials to hypotonic shock (osmotic stress) was investigated by exposing them to distilled water. Individual nematodes were washed three

times in distilled water before final transfer to distilled water in depression slides. After 1,3,6 and 24 hours of exposure the sex of the dead and/or burst individuals was recorded. Similar tests with L_2 showed no death or bursting within the 24 hours experimental period. Forty nematodes were used for each test.

b) Behavioural assay.

Tracks of 0, 4, 8, 16 and 24 days old nematodes grown individually were recorded photographically after they had been allowed to migrate over a thin layer of 4% Czapex dox agar in Pyrex Plastic Petri-dishes (diameter 5 cms), (Samoiloff, Balakanich and Petrovich, 1974). The nematodes were picked up with a brush joined to a pipette and placed at the centre of the Petri-dish. Tracks were recorded after 15 minutes. In the case of the 24 days old nematodes, the tracks were recorded after 30 minutes. Analysis of the tracks involved measuring the total linear distance travelled, number of turns (changes in direction), reversals and loops. Between 16 and 20 nematodes of each age were tested in each of two experiments (146 nematodes for the whole test).

c) Survival curves and fecundity.

The survival of nematodes grown either in pairs or individually in 0.2 ml medium was followed at room temperature (25°C). The investigation was carried out using the three supplements (ALE, ALE + Casein or 10% HLE) in SPYE. In the case of two nematodes per vial, three combinations were obtained - 2 males, 2 females or 1 male plus 1 female. In the latter case, the adults were transferred to new vials at the commencement of reproduction and the practice was continued until reproduction stopped. The off-spring were counted and discarded. The survival of 453 nematodes was followed in HLE supplemented medium and 100 in ALE and 76 in ALE + Cesein supplemented medium respectively.

To determine the effect of temperature increase on survival, 236 nematodes were grown individually in SPYE + HLE and incubated at 30 ± 0.85°C. As in the case of room temperature experiment each vial was examined every other day and the sex of dead nematodes was recorded.

C. Nutritional control of development

a) Variations in culture conditions were made in order to ascertain the role of the various components of the culture medium in growth and gonad development.

(i) SPYE alone.

Second stage larvae obtained as described previously, were washed 5-6 times in sterile distilled water and introduced into 1 ml of unsupplement SPYE. At two days intervals for a period of 14 days samples from these cultures were placed on a slide, air dried and stained with propionic-orcein(Boroditsky and Samoiloff, 1973).

The slides were examined within 24 hours of staining. The total body length and stage of gonad development of each nematode was recorded using the values obtained by Boroditsky and Samoiloff (1973).

Control experiments were carried out using SPYE + HLE grown nematodes. Since reproduction commenced within 5-6 days of incubation, the control experiments were terminated after 6 days. At least 100-150 nematodes were used at each time interval and the experiment was repeated three times.

(ii) Salts + HLE

This series of experiments were carried out using second stage larvae in 10% HLE in autoclaved salt solution consisting of 0.075 gms each of $MgSO_4$ and K_2HPO_4, 0.275 grams of NaCl and 0.3 gms of KNO_3 in 90 mls of distilled water (Dougherty, 1960).

The same procedure of staining and microscopic examination was used as described previously.

Control experiments were carried out with nematodes introduced into unsupplemented salt solution. Both experimental and control tests were repeated three times using at least 100 nematodes at each time interval.

b) Addition of missing medium components at various time intervals.

(i) At intervals of 4, 8 and 12 days, samples of nematodes previously maintained in salt solution + HLE or salt solution alone were washed and transferred to SPYE + HLE. At intervals of 2 days after transfer, the body length and stage of gonad development of at least 100 nematodes were examined after staining in propionic orcein. The practice was continued until reproduction commenced. Since most of the nematodes in salt alone died before the 12 day period, several samples had to be pooled together at the time of transfer. Counts of the number of offspring produced on the first day larvae were observed were made. The experiment was repeated three times.

(ii) Second stage larvae were washed and introduced into each of twelve 10 ml screw capped tubes containing 0.9 ml SPYE. After 4, 8 and 12 days, 0.1 ml

HLE was added to three of the tubes at each time interval. At two day intervals after the addition of HLE one of the samples was stained and examined microscopically as described previously. This was continued until reproduction commenced. The experiment was repeated three times.

D. Survival following nutritional stress

Nematodes that had been maintained for 4, 8 and 12 days in unsupplemented SPYE were transferred individually in 0.2 ml SPYE + HLE. At two day intervals, the vials were examined and the sex of the nematodes was determined.

Control experiments were carried out by maintaining the nematodes individually in unsupplemented SPYE throughout their life span. In most cases it was difficult to determine the sex of dead nematodes by direct microscopic examination. Such nematodes were stained in propionic orcein and microscopically examined.

Results

A. Population growth

Populations of up to 35,000-40,000 nematodes per ml was obtained within three weeks from an initial population of 1,000-1,500 second stage larvae per ml. After three weeks, a gradual decline in the number of living individuals occur (Figure 1 and Table 7 in appendix).

B. Assay for aging

a) Response to hypotonic shock.

The relationship between age and response to hypotonic environment (distilled water), is shown in Figure 2, Table 8 (appendix). As a function of age, the percentage of dead and burst nematodes increases and this effect is sex specific with males showing 50% dead/burst at day 16 and females at day 22. More nematodes of both sexes died than burst within the time limit of exposure.

b) Behavioural assay.

Behavioural changes as measured by the total distance travelled, the number of turns and reversals show three characteristics: (i) larvae and adults behave

differently, (ii) the behavioural activity of adults remains at a fairly constant level up to day 16 after which a sharp drop occurs, and (iii) there is no sex related difference in total activity at any of the ages tested (appendix, Figure 3 and Table 9).

The number of loops showed no significant change either in terms of stage, sex or age within the time limit of the test.

c) Survival and larval production.

Nematodes maintained in HLE, ALE or ALE + Casein supplemented medium have different mean survivals (Table 1 and appendix Figure 4). Under the three conditions, males did not live as long as females. Those that failed to mature (immature forms) had a lower survival time than adults except males in ALE +Casein supplemented medium.

In HLE supplemented medium, virgins of both sexes live longer than mated (Table 1 and appendix Figure 4). There was no significant difference between the mean survival of mated and virgin adults of both sexes in ALE supplemented medium. In ALE + Casein supplemented medium, virgin females live longer than mated but there was no significant difference between mated and virgin males (Table 1).

Increase in temperature to 30°C results in a decrease in mean survival time of males, females and immature forms as compared to 25°C (Table 1 and Figure 5 in appendix).

At 25°C in HLE supplemented medium, males and females occur in approximately equal proportions with 3.31% of the total failing to mature (Table 2). Increase in temperature to 30°C does not affect the percentage of males (when compared to 25°C) but a significant decrease in the percentage of adult females and a corresponding increase in the percentage of immature forms occurs (Table 2).

In both ALE and ALE +Casein supplemented medium, there was a great predominance of immature forms (Table 2).

Nematodes maintained in the three media conditions showed differences in generation time, mean larval production and the number of days before larval production ceased (Table 3). Under the three conditions, there was a gradual decline in the mean larval production as a function of time. In HLE supplemented medium, reproduction commenced 4 days after innoculation and continued up to day 20 (Table 3 and see appendix Figure 6). In ALE

supplemented medium, the generation time was between 6 and 8 days and larval production ceased 10 days after commencement. The generation time in ALE + Casien supplemented medium was between 4 and 6 days and larval production ceased at day 18.

C. Nutritional control of development

a) Nematodes maintained in salt solution alone did not grow (see appendix Figure 7) and showed no gonad development beyond the second stage. In salt solution + HLE, there was very limited growth and only 3.27% had third stage gonads, none of which show sex specificity (Figure 7). No morphological abnormalities were observed in either unsupplemented salts or salts + HLE.

In SPYE alone, males and females grow to roughly the same length. This length corresponds to that of the moult from fourth stage larva to adults (Boroditsky and Samoiloff, 1973). In control SPYE + HLE, females grow more than males (see appendix Figure 7 and Table 10).

The effect of SPYE alone on gonad development varies. About ninety-six percent of the male stages had normal adult gonads after 8-10 days. However after 10 days, the gonads began to show morphological abnormalities and failed to take up stain. Figures 9 and 10 show gonads

of males in SPYE + HLE after 4 days and male from SPYE alone after 8 days respectively. The male gonad characteristic that could be easily identified after 12-14 days are the spicules. The effect on the female development was more variable. Only 35.2% show adult female characteristics by day 14 and of these 32.5% were abnormal (see appendix Figure 8 and Table II). The commonest abnormalities were the development of two vulvas (Figure 13) and formation of cuticular blisters (Figure 14). Figure 11 and 12 show normal adult female gonad from SPYE + HLE at day 4 and female from SPYE alone at day 10 respectively.

b) Nematodes transferred from either salt solution or salts + HLE after 4, 8 and 12 days developed normal gonads, attained adult size and reproduced within 6 days in all cases (Figure 15 and Table 12 in appendix). Addition of HLE to nematodes maintained in SPYE alone for 4, 8 and 12 days results in commencement of reproduction within 4-6 days after addition. However, they failed to reach normal adult size before commencement of reproduction (appendix Figure 16).

The mean larval production per female on the first day of reproduction by nematodes transferred from salts to SPYE + HLE at days 4, 8 and 12 were 9.03, 8.80

and 6.83 respectively, while those transferred from salts + HLE were 6.05, 8.02 and 9.36 respectively. In SPYE alone for 4, 8 and 12 days, the mean larval production at the commencement of reproduction were 7.09, 3.95 and 4.05 per adult female respectively. In SPYE + HLE, the mean larval production by day 6 was 27.74.

Table 4 shows a summary of the general effects of various levels of nutrition on growth and gonad development and the general effect of the removal of stress at various time intervals.

D. Survival following nutritional stress

Nematodes maintained in SPYE alone throughout their life span were so abnormal that in most cases it was not possible to determine their sex. Adult males could be identified by the presence of spicules. Sixty percent of the nematodes could not be sexed. The remaining 39.45% that could be identified as males had a longer mean survival than males in SPYE + HLE. The abnormals had a mean survival time similar to females in SPYE + HLE (Table 1 and 5; appendix Figures 4, 17 and 18).

The mean survival time of nematodes transferred from SPYE to SPYE + HLE at days 4, 8 and 12 were greater

than those maintained in SPYE + HLE under the same conditions (25°C) (Tables 1 and 5; appendix Figures 4, 17 and 18). In all cases, males did not live as long as females and those that failed to mature lived a shorter time than adults. No difference in the mean survival of immature forms transferred from SPYE or those in SPYE + HLE throughout was observed (Tables 5 and 1, Figures 4, 17 and 18).

The percentage sex distribution of nematodes transferred after 4 and 8 days in SPYE, does not differ significantly but the percentage of immature forms increased as compared to those in SPYE + HLE (Tables 2 and 6). In the case of nematodes transferred after 12 days in SPYE, the percentage of males was not different from that seen in SPYE + HLE but there was a significant drop in the percentage of females and an increase in the percentage of immature forms. Nematodes transferred after 4 and 8 days, developed normal gonads with no morphological abnormalities while 20.45% of the females and 7.14% of the males transferred from 12 days had abnormalities respectively.

Discussion

The results of this study show that the aging pattern of *P. redivivus* is basically similar to that reported for the free-living nematode, *T. aceti* (Gershon, 1970); and *C. briggsae*, (Zuckerman, et. al., 1971). The pattern is characterized by growth and reproduction culminating, as a function of time, in death. As the nematodes age, they become more susceptible to hypotonic shock and exhibit changes in their activity.

The increased susceptibility to hypotonic shock is in agreement with the report of Zuckerman, et. al., (1971); with *C. briggsae*. The increased susceptibility of *C. briggsae* with age, was suggested to be due to time dependent changes in the cuticle in the form of degeneration of the hypodermal mitochondria, thickening of the hypodermal band, formation of lysosome-like bodies in hypodermis, separation of osmophilic membrane from the cuticle and the accumulation of electron-dense materials and electron-dense balls in the fluid layer, (Zuckerman, et. al., 1971, 1972 and 1973). Although no such age related studies has been reported for any free-living diecious nematode, it is possible that the ultrastructural changes follow basically the same pattern.

Behavioural activity measured by the number of head swings has been reported to decline as a function of age in *C. briggsae*, (Zuckerman, et. al., 1971). In this study, behavioural activity shows a stage specific pattern but the adults show a fairly constant behavioural level up to day 16 after which a sharp drop occurs. The implications of this is unclear but it is of significance to note that the drop occurs at about the point at which sexual activity, as measured by larval production, ceases (Table 3). Whether this is a casual relationship is not clear but poses a problem for further investigation. It can also be argued that the intervals between measurements might have been too long to detect any differences. No difference has been found between the behaviour of early adults of *P. redivivus* although larvae behave differently from adults (Carol Pollock - personal communication). This could explain the lack of sexual differences in activity.

The diecious nematode *T. aceti*, has a mean life expectancy of 25 days (Gershon, 1970) and this is not altered by inhibitors of DNA synthesis. The hermaphroditic nematode *C. briggsae* lives for an average of 28-30 days at 25°C and increase in temperature to 27° results in a decrease in mean life expectancy to

26 days (Zuckerman, et. al., 1971). The result of this study shows that *P. redivivus* at 25°C under the same nutritional conditions used for *C. briggsae* and *T. aceti*, has an average life span of 25-36 days and that at 30°C, the mean life expectancy drops to 18-25 days (Table I). When the composition of the medium is changed by replacing HLE with ALE or ALE +Casein, a significant decrease in the mean life expectancy occurs. The significance of this lies in the fact that it suggests that survival time is a function of the type of nutrient. This interpretation should however, be made with caution particularly because cultures maintained in medium supplemented with autoclaved liver extract require more constant re-innoculation than cultures with HLE as supplement. This suggests the possibility that ALE has some detrimental effects on the nematodes.

The most significant factor that has emerged from the aging assay studies of *P. redivivus* is the wide degree of sexual dimorphism exhibited. Firstly males are more susceptible to hypotonic shock than females of the same chronological age. Secondly, males have a lower mean survival time than females regardless of nutritional or temperature conditions. Thirdly, adults live longer than those that failed to mature. It is therefore

conceivable to conclude that nematode aging is a genetically programmed developmental phenomenon which is both sex and stage specific.

Sex specific differences in nematode development has been reported for several species of nematodes. In the diecious nematode *P. silusiae*, there is no structural difference in the cuticle of males and females but the moulting pattern is both stage and sex specific (Samoiloff and Pasternak, 1968). Laser microbeam irradiation studies has revealed that irradiation of the region just anterior to the germinal primordium of *P. silusiae*, produces a significant increase in the proportion of females due to blocking of male gonad development which normally grows anteriorly (Samoiloff, 1973). A combination of high temperature and low protein inhibits the development of female *P. redivivus* without any effects on the males (Hansen and Cryan, 1966). Males develop at 30°C or in presence of ethanol in axenic cultures of the parthenogenetic nematode *Aphelenchus avenea* and in monoxenic cultures with the fungus *Rhizoctonia solani*. This masculinizing effect is prevented by mitomycin C (Hansen, Buecher and Yarwood, 1973). High temperatures influence both the developmental rate and sex ratio of the plant parasitic nematodes

Meloidogyne graminis and *Hyposperin graminis* with males predominating at 32°C and virtually absent at lower temperatures (Laughlin, Williams and Fox, 1969; Laughlin and Williams, 1969). In the hermaphroditic nematode *C. briggsae*, 75% of adults are males under conditions which cause most of the hermaphrodites to go into dauer stage, this causing an increase in male-to-hermaphrodite ratio (Yarwood and Hansen, 1969). In fecal cultures of the parasitic nematode *Strongyloides fullerboni*, thickness of medium and temperature control the development of sexual forms with males and filariforms appearing in thin smears and female and filariforms predominating in heavy smears over a range of temperature conditions (Hansen, Buechen and Cryan, 1969).

Further support for sex specificity of nematode development is shown by the nutritional effects of this study. Elimination of the protein supplement (HLE), from the medium, results in the growth of both sexes to about equal size (which is about half the size of normal adults), (Boroditsky and Samoiloff, 1973). However, examination of the gonads revealed that by 8-10 days, ninety-six percent of the males have developed adult gonads while only a small proportion of the females had reached adult stage. This result shows

conclusively that the two sexes differ in their nutritional requirements in so far as gonad development is concerned. It also agrees with an earlier report by Hieb and Stokstad, (1970); who found that *C. briggsae* in the basal medium grows to the size of fourth stage larvae or small adults without reproduction.

Several of the abnormalities observed by growing the nematodes in the basal medium alone, mimics the effects of chemical inhibitors of DNA synthesis observed in *P. silusiae* (Westgarth-Taylor and Pasternak, 1973); in *P. redivivus* (Boroditsky and Samoiloff, 1973); and in *C. briggsae* and *T. aceti* (Kisiel, et. al., 1972). The general effects of inhibitors of DNA synthesis has been the occurrence of cuticular blisters and lack of differentiation of gonads although there is cell proliferation. The basic difference between the effects of inhibitors of DNA synthesis and the effects obtained by growing the nematodes in unsupplemented SPYE however, is that the abnormalities have been restricted mainly to the females. The effect of inhibitors has not been linked with the induction of cuticle blisters but rather, it has been suggested that a relationship exists between the presence of gonad tissue and the normal pattern of cuticle

biosynthesis in adult worms (Westgarth-Taylor and Pasternak, 1973). While this relationship is speculative, it ties in with the results obtained from this study with females showing a high degree of germ cell proliferation without differentiation into gonads and possessing cuticle blisters. Further, a developmental link between mating attraction and gonad development has been reported in *P. silusiae* (Cheng and Samoiloff, 1972). The mating attraction system (production of attractant by females and the response to attractant by males), does not occur in nematodes treated with hydroxyurea.

The fact that salt solution alone or salt supplemented with 10% HLE support no growth or gonad development is interesting. It supports earlier reports that the importance of HLE or protein supplement lies in maturation and reproduction, (Sayre, et. al., 1963; Rothstein and Cook, 1965; Jackson, 1969; Hieb and Rothstein, 1968; Cole and Dutky, 1968; Buecher, Hansen and Yarwood, 1970a and 1970b).

The immediate increase in growth and gonad development followed by reproduction of nematodes transferred from salt solution or salts + HLE to SPYE + HLE shows that lack of growth and gonad differentiation

does not affect the ability of the nematodes to recover and continue normal postembryonic development in presence of optimal conditions. With nematodes maintained in SPYE, there is partial growth and differentiation and in some cases adult stages of gonad development. Thus, lack of attainment of full adult size before reproduction commences suggests a partial loss of ability to grow fully with no effect on reproduction. However, this interpretaion should be made with caution since the measurements were stopped at commencement of reproduction. It is still interesting that the recovery rate after partial differentiation is far less than in the case where no differentiation or gonad development has occurred.

One of the basic features of maintaining nematodes in unsupplemented SPYE, is the retardation of developmental rate. Nematodes of different species have been held under adverse physical and physiological conditions and have been shown to recover and undergo the normal patterns of development when returned to optimal conditions (Hansen and Cryan, 1966; Cooper and Van Gundy, 1970; Jackson, 1973). One feature of this study has been the development of structural abnormalities coupled with retarded growth rate. Despite these

abnormalities, the mean life expectancy can be extended. There is no evidence that return of the nematodes to optimal conditions has any influence on ability to repair abnormal parts. In fact this is not the case since a large proportion of the nematodes transferred from unsupplemented SPYE to SPYE + HLE after 12 days still had abnormalities. The most significant point therefore, is that developmental rate plays a significant role in determining how long *P. redivivus* lives.

Several other factors that affect the development rate also affect the mean life expectancy and sex ratio. At 30°C, the nematodes attain sexual maturity much faster than at 25°C and have a shorter mean survival time. There were also more adult males. The type of nutrient affects not only the mean larval production but also the survival time. This is shown by differences obtained in the mean larval production (Table 3) and survival of nematodes maintained in HLE, ALE and ALE + Casein supplemented medium. In all cases (temperature and nutrition), the survival time is sex specific with males living much shorter than females. Sexual activity which does not affect the developmental rate, affects the mean life expectancy as shown by the fact that mated nematodes of both sexes do not live as long as virgins.

This study has described the aging pattern of *P. redivivus* and the role of developmental rate in determining the survival pattern. In several respects it agrees with earlier findings on aging of some free-living nematode species. However, it points to a wide degree of sexual dimorphism in nematodes and raises the question of their use as model organisms for aging studies.

The basic idea of using nematodes as model organisms for aging studies, is to seek answers concerning the mechanisms and molecular basis of the aging process. These are based on the contention that while the structural and functional features may senescence in a peculiar way, the basic mechanisms involved in aging are possibly the same in all animals.

From the theoretical point of view, several theories of aging have been proposed. These approaches are based on the common assumption that aging represents a loss and deterioration of biological information which occur at the cellular level and depends upon time and/or metabolic level (Comfort, 1970, Medvedev, 1972). As argued by Comfort, (1970); if the loss occur at the cellular level "it becomes necessary to decide if it is predominantly in cells such as neurons,

or in clonally dividing cells and whether the leading process in either case is one of noise accumulation in homoestatic and copying processes or whether it is secondary to differentiation, depending on the irreversible switching off of synthetic capacities with morphogenesis". Thus, any model of information loss whether cellular, molecular or intercellular, requires evidence of non-sense cells or materials accumulating with age. Based on these arguments, Comfort (1970); classified the various theories of aging into two categories: "primary error" hypothesis and non-DNA error theories.

The "primary error" hypothesis proposes that information is being lost from DNA either through mutation, macromolecules damage or epigenetic masking. Error accumulation in DNA has not been exclusively demonstrated and it cannot exclude the possibility of the error(s) occurring at post-DNA level, e.g., nuclear structures other than molecular DNA or later transcription or translation steps as proposed the non-DNA error theories.

One of the implications of the "primary error" hypothesis, is that the information is being lost upon DNA replication. In nematodes, this

cannot be the case as it has been demonstrated that inhibition of DNA synthesis does not alter developmental rate in tissues other than the reproductive tissue (Pasternak and Samoiloff, 1970; Boroditsky and Samoiloff, 1973). Furthermore, inhibition of DNA synthesis according to Gershon, (1970); does not alter the mean life expectancy, while Kisiel, et. al., (1972); demonstrated a decrease in life expectancy with DNA synthesis inhibition. The latter observation is in direct opposition to the prediction of the primary error hypothesis.

While non-DNA mechanisms of information loss cannot be excluded, a more likely mechanism for aging in the nematode involves the expression of a series of genes producing the syndrome for senescence. This hypothesis is based on the stage and sex specificity of senescence shown by this study and the relationship between senescence and post embryonic development rate and the fact that factors like nutrition and temperature which control developmental rate, also control the life expectancy. It would appear that in the normal course of nematode post embryonic development, the onset of aging is triggered by a specific developmental event. The results presented here would tend to indicate that the timing of this trigger coincides with the completion of the final moult.

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Table I. Effect of various protein supplements on mean survival.

Nutritional Level	Mean Survival (± S. E.) - Days.			
		Males	Females	Immature
SPYE + HLE (25°C)	Virgin :	29.26 ± 0.83	36.39 ± 0.82	23.75 ± 1.94
	Mated :	25.65 ± 1.01	31.94 ± 1.48	-
SPYE + HLE (30°C)	Virgin :	20.90 ± 0.65	24.46 ± 0.58	18.83 ± 1.01
	Virgin :	21.03 ± 1.17	29.21 ± 1.71	17.17 ± 1.53
	Mated :	19.69 ± 1.72	28.15 ± 2.01	-
	Virgin :	23.25 ± 1.95	31.90 ± 1.27	20.53 ± 1.54
	Mated :	23.83 ± 2.25	26.17 ± 2.83	-

S. E = Standard Error.

Table 2. Effect of various protein supplements on % sex distribution.

Nutritional Level	Percentage		
	Males	Females	Immature
SPYE + HLE (25°C)	48.79	47.90	3.31
SPYE + HLE (30°C)	46.19	38.56	15.25
SPYE + ALE (25°C)	46.00	42.00	12.00
SPYE + ALE + Casein (25°C)	39.22	42.05	18.73

Table 3. Effect of various supplements on mean Larval production*.

Days after commencement of reproduction	Nutritional Level		
	SPYE + HLE	SPYE + ALE	SPYE + ALE + Casein
0	2.99 ± 0.54	0.93 ± 0.73	1.33 ± 0.93
2	17.13 ± 1.62	6.62 ± 2.70	13.77 ± 3.31
4	13.99 ± 1.15	9.83 ± 3.18	13.75 ± 2.71
6	8.04 ± 1.25	7.23 ± 3.26	2.71 ± 1.51
8	3.79 ± 1.91	2.31 ± 1.34	1.67 ± 0.80
10	2.85 ± 0.92	-	-
12	1.53 ± 0.73	-	
14	0.38 ± 0.29	-	
Total	50.78 ± 3.27	26.92 ± 5.99	41.39 ± 5.70

* ± Standard error (S. E.).

Table 4. Summary of the effect of various nutritional Levels on growth and gonad development.

Nutritional Level		
SPYE + HLE	Normal	Reproduction in 5 days
SPYE alone	Limited	Male - Normal unstable. Female - immature abnormal.
SPYE (4, 8 and 12 days) transfer to SPYE + HLE	Limited	Normal with reproduction 4 -6 days after transfer.
Salts alone	None	None
Salts + HLE	very limited	None
Salts alone or salts + HLE (4, 8 and 12 days) transfer to SPYE + HLE	Normal	Reproduction within 10 days after transfer.

Table 5. Effect of addition HLE at various times on mean survival.

Nutritional Level	Mean survival time ± S. E. (days).		
	Males	Females	Immature
SPYE alone	34.42 ± 1.57	36.58 ± 1.32	-
SPYE (4 days)	33.47 ± 1.57	39.06 ± 1.61	23.00 ± 3.54
SPYE (8 days)	38.42 ± 1.80	44.73 ± 1.74	24.00 ± 1.42
SPYE (12 days)	40.29 ± 1.56	47.00 ± 1.64	26.44 ± 1.47

Table 6. Effect of addition of HLE at various times on % sex distribution (25°C).

Nutritional Level	Percentage		
	Males	Females	Immature
SPYE alone	39.45	60.55*	-
SPYE (4 days)	44.54	46.37	9.99
SPYE (8 days)	44.83	45.08	9.47
SPYE (12 days)	47.46	37.29	15.25

* abnormal animals.

APPENDIX

Table 7. Table of Population Growth

Time (Days after innoculation)*	Mean number of nematodes** on T/V screen					Approximate number of worms 1 ml. (X 10^3).
	Exp't 1	Exp't 2	Exp't 3	Total	Mean	
0	15	10	14	39	13.00	1.44
2	8	8	16	32	10.67	1.19
4	13	17	14	44	14.67	1.63
6	24	26	35	85	28.33	3.15
8	76	102	96	274	91.33	10.01
10	83	134	120	337	112.33	12.15
13	207	231	203	641	231.67	23.74
15	170	276	208	654	218.00	24.22
20	270	228	242	740	246.67	27.41
22	328	306	276	910	303.33	33.70
24	306	283	289	878	292.67	32.52
26	294	352	315	961	320.33	35.59
28	302	332	320	954	318.33	34.58
30	247	274	254	775	258.33	28.70
32	207	246	289	742	247.33	27.48

* Reproduction commenced between 4 and 6 days after innoculation.

**approximate volume of medium screened or T/V screen = 0.009 ml.

Table 8. Age related differences in response to osmotic stress.

Age (Days)	Sex	Exposure Time (Hours)											Percentage Dead/ Burst
		1		3		6		24					
		D*	B*	D	B	D	B	D	B	D	B	Total	
4	M** (21)	1	0	1	0	1	0	0	1	3	1	4	29.04
	F** (19)	0	0	0	0	0	0	1	1	1	1	2	10.53
8	M (18)	0	0	0	0	3	0	4	1	7	1	8	44.44
	F (21)	0	0	0	0	1	0	2	0	3	0	3	14.28
16	M (16)	0	1	0	4	0	0	3	0	3	5	8	50.00
	F (20)	0	1	1	1	0	0	1	1	2	3	5	25.00
24	M (21)	7	1	2	1	3	0	1	1	13	3	16	76.19
	F (24)	1	2	2	2	5	0	2	0	10	4	14	58.33

D = dead, B = Burst, F = Females, M = Males.

Numbers in bracket represent number of nematodes tested.

Table 9. Analysis of behavioral changes with age.

Age (Days)	Sex	No.	Distance	Reversals	Turns	Loops
0	L_2	15	9.10 ± 1.18	4.73 ± 0.62	7.07 ± 1.18	2.80 ± 0.72
4		17	38.18 ± 6.70	19.12 ± 2.55	29.06 ± 5.48	2.40 ± 0.71
		18	29.33 ± 5.10	12.72 ± 1.60	26.11 ± 3.63	3.94 ± 1.19
8		19	24.45 ± 3.48	9.11 ± 1.42	22.48 ± 3.33	1.63 ± 0.54
		20	25.45 ± 3.82	13.35 ± 1.46	23.45 ± 3.62	2.26 ± 0.64
16		14	24.28 ± 5.23	13.92 ± 1.91	31.29 ± 4.07	1.78 ± 0.49
		14	23.46 ± 3.85	11.85 ± 1.38	28.07 ± 4.36	1.00 ± 0.32
24		13	3.46 ± 0.87	2.65 ± 0.59	7.04 ± 1.39	0.27 ± 0.11
		16	5.91 ± 0.86	4.06 ± 0.90	9.75 ± 1.38	0.25 ± 0.14

Table 10. Effect of various levels of nutrition on growth (length in microns.

Time* (Days)	SPYE + HLE**		SPYE alone		Salt + HLE	Sale alone
	Males	Females	Males	Females	-	-
2	634 ± 6		488 ± 7		434 ± 10	369 ± 10
4	1149 ± 26	1191 ± 28	682 ± 17		443 ± 7	374 ± 7
6	1352 ± 50	1660 ± 64	852 ± 39	832 ± 38	450 ± 12	358 ± 5
8	-	-	790 ± 33	803 ± 21	473 ± 11	368 ± 10
10	-	-	827 ± 21	783 ± 28	494 ± 10	338 ± 8
12	-	-	760 ± 22	776 ± 21	473 ± 11	355 ± 9
14	-	-	771 ± 21	794 ± 18	470 ± 15	327 ± 9

* Length at = 350 ± 7 (microns)

** Reproduction commences between day 4 and 6.

Table 11. % distribution of male and female stages of gonad development in SPYE with or without HLE.

Time (Days)	Stage of gonad development	SPYE		SPYE + HLE	
		Male	Female	Male	Female
4	L_3	30.99	93.42	3.33	13.40
	L_4	69.10	6.58	5.00	19.40
	Adult (Normal	-	-	91.67	67.20
	Adult (Abnormal	-	-	-	-
6	L_3	22.34	64.85	-	-
	L_4	66.28	35.15	3.38	6.41
	Adult (Normal	11.38	-	96.62	93.59
	Adult (Abnormal	-	-	-	-
8	L_3	0.99	68.13		
	L_4	21.78	23.10		
	Adult (Normal	75.25	2.18		
	Adult (Abnormal	1.98	6.59		
10	L_3	-	8.11		
	L_4	3.70	75.68		
	Adult (Normal	85.19	5.40		
	Adult (Abnormal	11.11	10.81		
12	L_3	0.86	6.99		
	L_4	3.31	65.06		
	Adult (Normal	58.72	3.49		
	Adult (Abnormal	37.11	24.06		
14	L_3	0.85	9.77		
	L_4	3.34	55.02		
	Adult (Normal	41.30	2.71		
	Adult (Abnormal	55.51	32.50		

Table 12. Effect of transfer from various nutritional levels to SPYE + HLE on growth (length in microns)*

Days before transfer	Days after transfer								
	SPYE alone			Salts + HLE			Salts alone		
	2	4	6	2	4	6	2	4	6
4 (M)	869 ± 12	1053 ± 20	-	731 ± 23	1244 ± 27	-	687 ± 17	1230 ± 32	-
(F)	945 ± 38	1219 ± 33	-	786 ± 26	1488 ± 41	-	714 ± 22	1507 ± 51	-
8 (M)	886 ± 21	842 ± 27	-	820 ± 24	1247 ± 30	-	623 ± 35	988 ± 43	1251 ± 32
(F)	949 ± 30	1010 ± 31	-	840 ± 33	1461 ± 43	-	661 ± 31	1273 ± 56	1543 ± 51
12 (M)	803 ± 16	848 ± 16	911 ± 24	810 ± 24	1239 ± 25	-	508 ± 27	988 ± 35	1241 ± 44
(F)	854 ± 23	950 ± 35	1066 ± 36	835 ± 49	1539 ± 35	-	636 ± 26	1083 ± 43	1570 ± 51

* Measurements were stopped at the commencement of reproduction.

F = Females, M = Males.

Table 13. Breakdown of survival in SPYE + HLE at room temperature (25°C)

Time Range (Days)	MALES				FEMALES				IMMATURE	
	Virgin		Mated		Virgin		Mated		-	
	No.	% survival	No.	% survival	No.	% survival	No.	% survival	No.	% survival
8 - 12	-	-	3	96.74	-	-	7	92.47	1	93.33
13 - 16	4	96.90	3	93.48	1	99.19	3	89.25	2	80.00
17 - 20	11	88.37	21	70.66	5	95.16	5	82.88	5	46.67
21 - 24	26	68.22	23	45.65	9	87.03	7	77.42	2	32.67
25 - 28	23	50.39	16	28.26	9	80.65	13	63.50	3	13.33
29 - 32	27	29.36	14	13.05	17	66.94	19	43.01	1	6.67
33 - 36	20	13.73	6	6.57	24	47.57	14	37.96	0	6.67
37 - 40	6	10.08	1	5.00	23	29.07	4	23.66	1	0
41 - 44	5	6.20	2	3.26	20	12.91	11	12.83	-	-
45 - 48	5	1.33	3	0	9	5.65	5	6.45	-	-
49 - 52	2	0.78	-	-	4	2.42	3	3.23	-	-
54 - 56	1	0	-	-	3	0	2	1.07	-	-
57 - 60	-	-	-	-	-	-		-	-	-
	129		92		124		93		15	

Table 14. Breakdown of survival at 30°C

Time of death range	Males		Females		Immature	
	No.	% survival	No.	% survival	No.	% survival
5 - 8	1	99.10	0	100.00	1	97.40
9 - 12	14	87.10	2	97.80	6	80.60
13 - 16	19	68.80	7	90.10	7	41.60
17 - 20	23	47.70	15	73.60	10	30.60
21 - 24	20	29.30	23	50.50	5	16.70
25 - 28	13	18.30	17	31.90	5	5.60
29 - 30	17	1.80	25	3.30	2	0
31 - 32	2	0	2	0	-	-
Total	109	-	91	-	36	-

Table 15. Breakdown of survival in SPYE.

Time Range (Days)	MALES		ABNORMALS	
	No.	% survival	No.	% survival
13 - 16	0	100.00	1	98.48
17 - 20	4	90.70	0	98.48
21 - 24	6	76.75	7	87.88
25 - 28	6	72.80	9	74.24
29 - 32	8	44.19	15	51.52
33 - 36	7	27.91	7	40.91
37 - 40	1	25.58	5	33.33
41 - 44	2	20.30	8	21.11
45 - 48	3	13.95	5	13.64
49 - 52	3	9.30	4	7.58
53 - 56	2	4.65	1	6.06
57 - 60	1	0	3	1.57
61 - 64	-	0	1	0
	43	-	66	-

Table 16. Breakdown of survival after 4 and pooled 8/12 days in HPYE alone.

Time range (Days)	4 days in SPYE						Pooled 8 and 12 days in SPYE					
	MALES		FEMALES		IMMATURE		MALES		FEMALES		IMMATURE	
	No.	% survival	No.	% survival	No.	% survival	No.	% survival	No.	% survival	No.	% survival
14 - 16	1	97.96	-		2	80.00	2	98.17	1	99.00	2	92.86
17 - 20	5	87.75	6	88.24	2	60.00	8	90.83	2	97.00	3	82.14
21 - 24	8	71.43	2	84.33	3	30.00	7	84.40	2	95.00	6	60.71
25 - 28	4	63.27	2	80.40	0	30.00	7	77.98	3	92.00	6	39.29
29 - 32	4	55.10	5	70.69	1	20.00	12	66.97	9	83.00	3	28.57
33 - 36	10	34.69	5	60.58	0	20.00	16	52.28	8	75.00	2	10.71
37 - 40	5	24.49	8	45.10	1	10.00	10	43.12	8	67.00	1	7.14
41 - 44	4	16.33	8	29.41	1	0	13	31.20	13	54.00	2	0
45 - 48	3	10.62	5	19.61	-	-	7	24.77	12	42.00	-	-
49 - 52	3	4.50	6	7.84	-	-	7	18.35	14	28.00	-	-
53 - 56	1	2.04	1	5.88	-	-	10	9.17	6	22.00	-	-
57 - 60	1	0	3	0	-	-	6	3.67	8	14.00	-	-
61 - 64	-	-	-	-	-	-	3	0.92	7	7.00	-	-
65 - 68	-	-	-	-	-	-	1	0	5	2.00	-	-
69 - 72	-	-	-	-	-	-	-	0	2	0	-	-
Total	49	-	51	-	10	-	109	-	100	-	25	-

Figure 1. Population growth at 25°C.

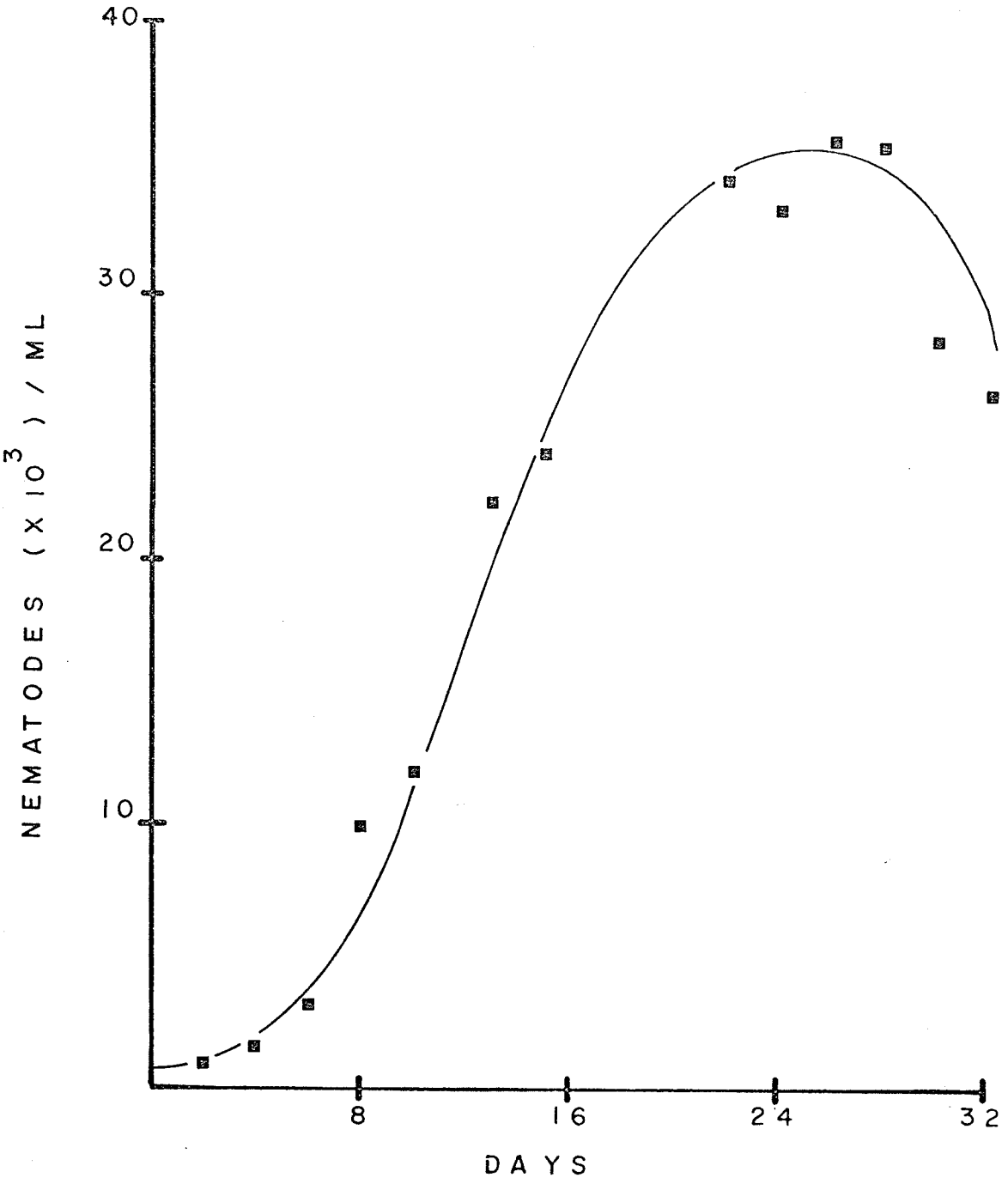

40
30
20
10
NEMATODES (X 10 3) / ML
8
16
24
32
DAYS

Figure 2. Age related differences in response to hypotonic shock (a - males, b - females).

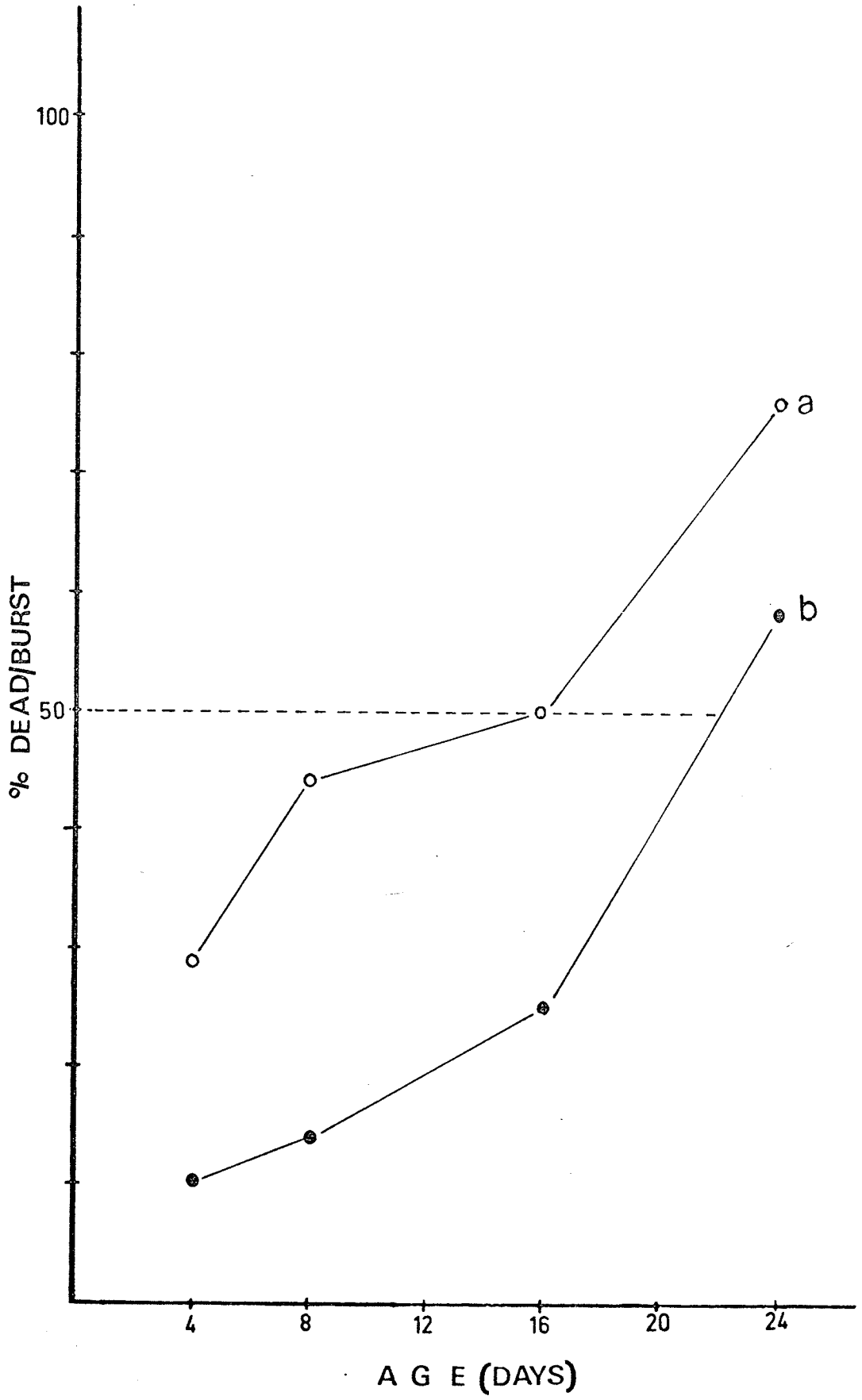

100
50
% DEAD/BURST
a
b
4
8
12
16
20
24
A G E (DAYS)

Figure 3a - d. Age related changes in behavioural activity (dotted - second stage larvae, plain - males, stripped - females).

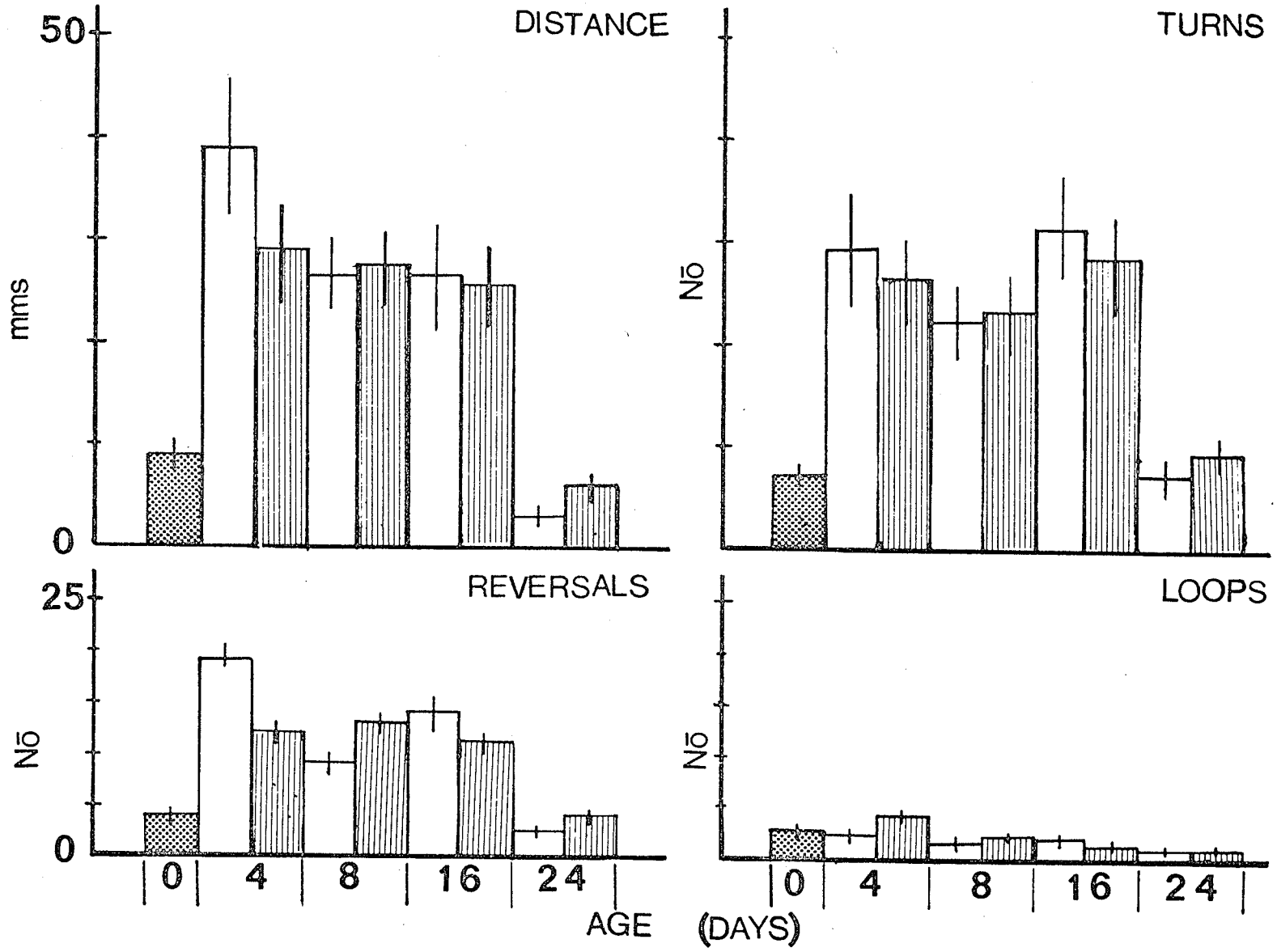

DISTANCE
TURNS
50
0
mms
No
REVERSALS
LOOPS
25
0
No
0
4
8
16
24
AGE
(DAYS)

Figure 4. Survival curves at room temperature (25°C) in SPYE + HLE (closed squares - immature forms, closed triangles - mated males, closed circles - virgin male, open triangles - mated females, open circles - virgin females.

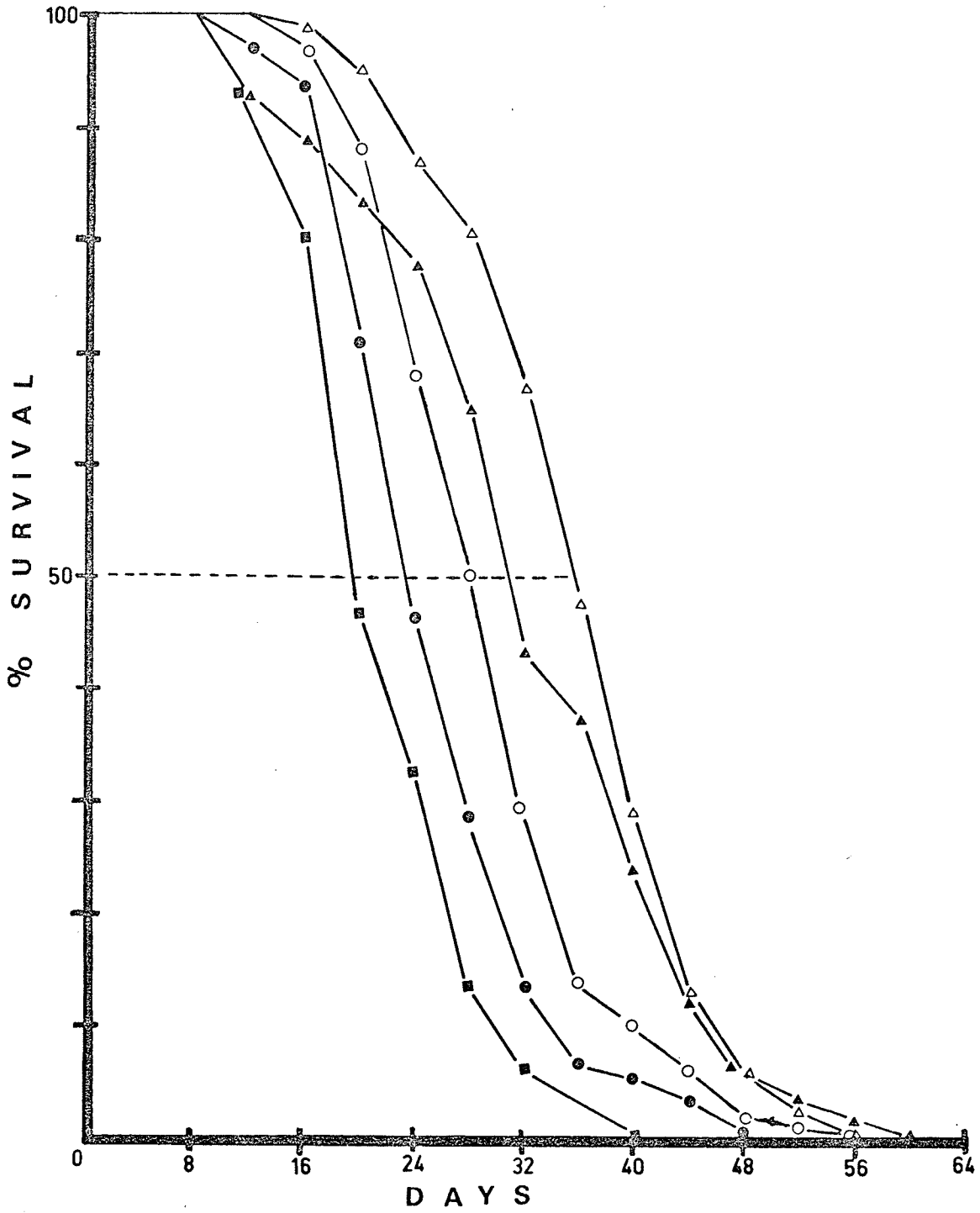

100
50
0
% SURVIVAL
8
16
24
32
40
48
56
64
DAYS

Figure 5. Survival curves at 30°C in SPYE + HLE (open triangles - immature forms, closed circles - males, open circles - females).

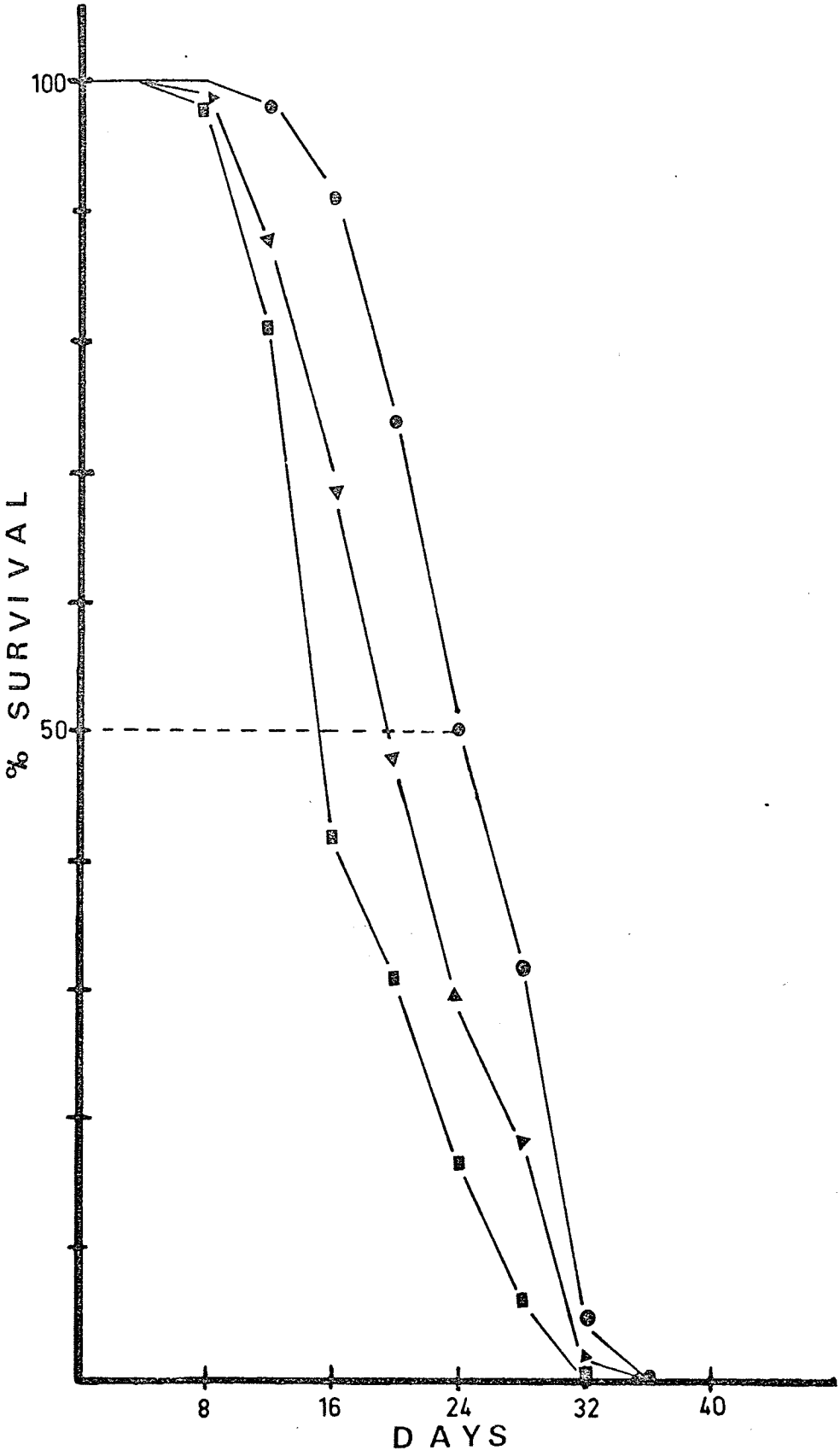

100
50
% SURVIVAL
8
16
24
32
40
DAYS

Figure 6. Mean larval production as a function of time in SPYE + HLE at 25°C.

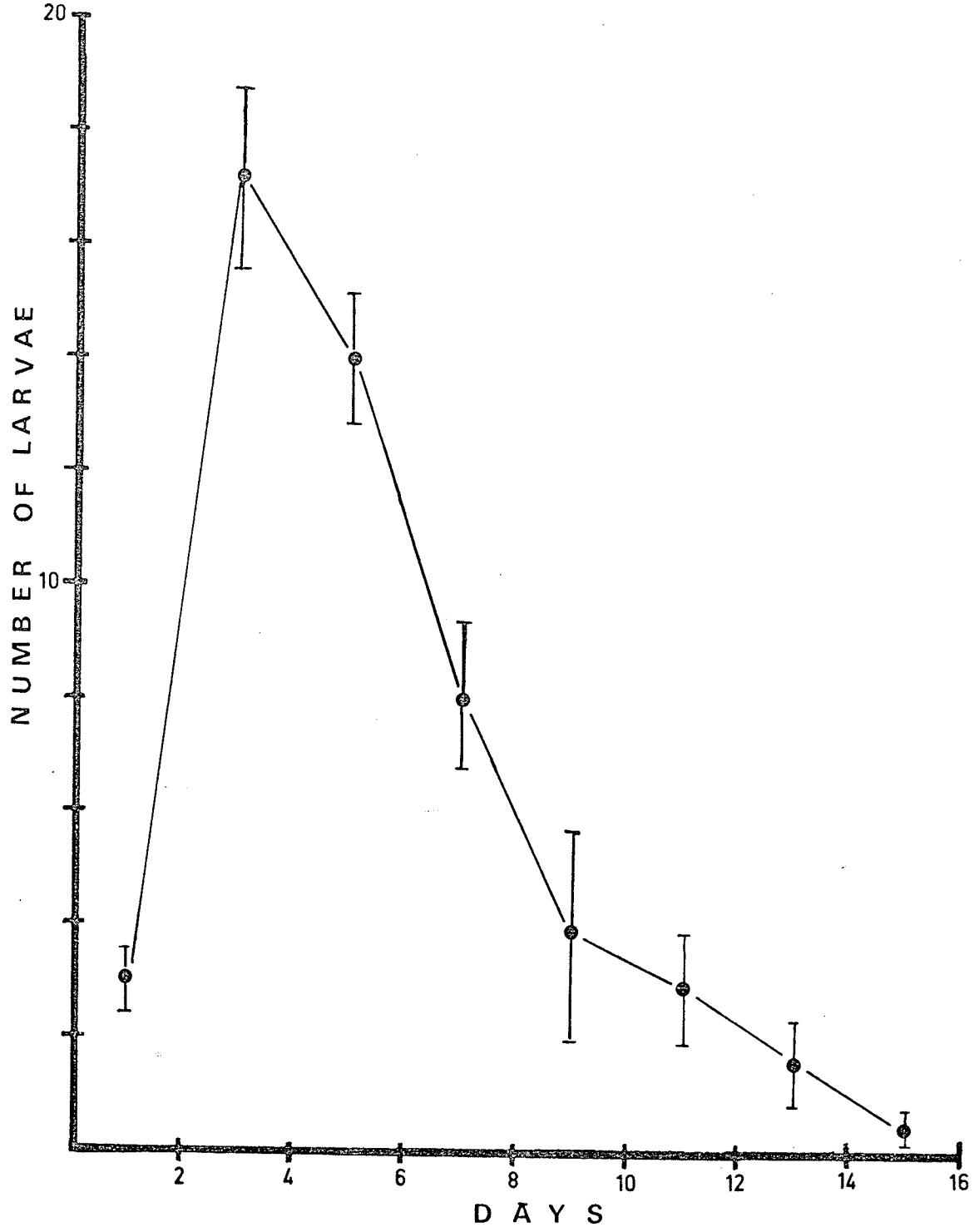

20
10
NUMBER OF LARVAE
2
4
6
8
10
12
14
16
DAYS

Figure 7. Growth curves under various nutritional levels (a - control females, b - control males, c - SPYE alone females, d - SPYE alone males, e - salts _ HLE and f - salts alone).

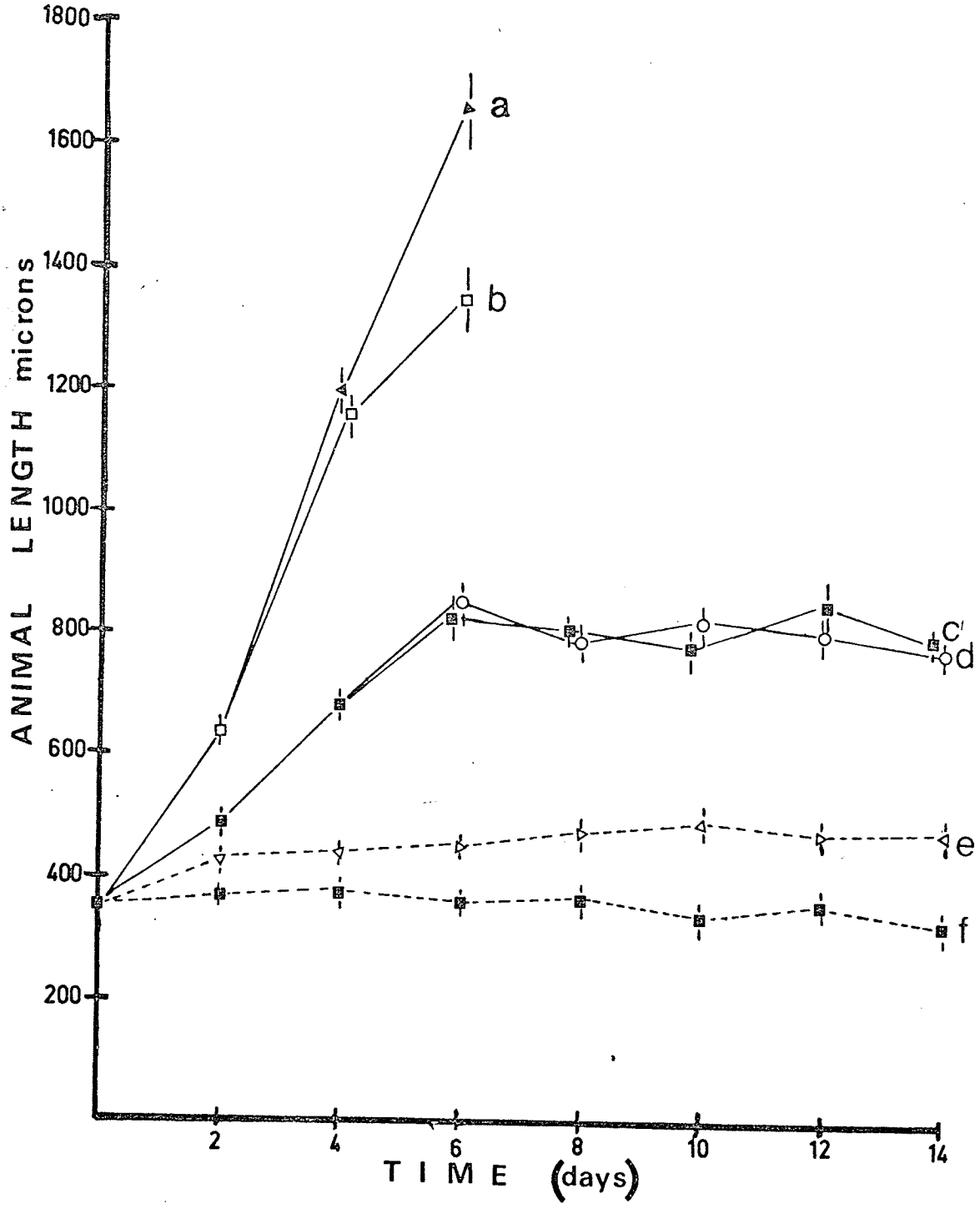

1800
1600
1400
1200
1000
800
600
400
200
ANIMAL LENGTH microns
a
b
c
d
e
f
2
4
6
8
10
12
14
TIME (days)

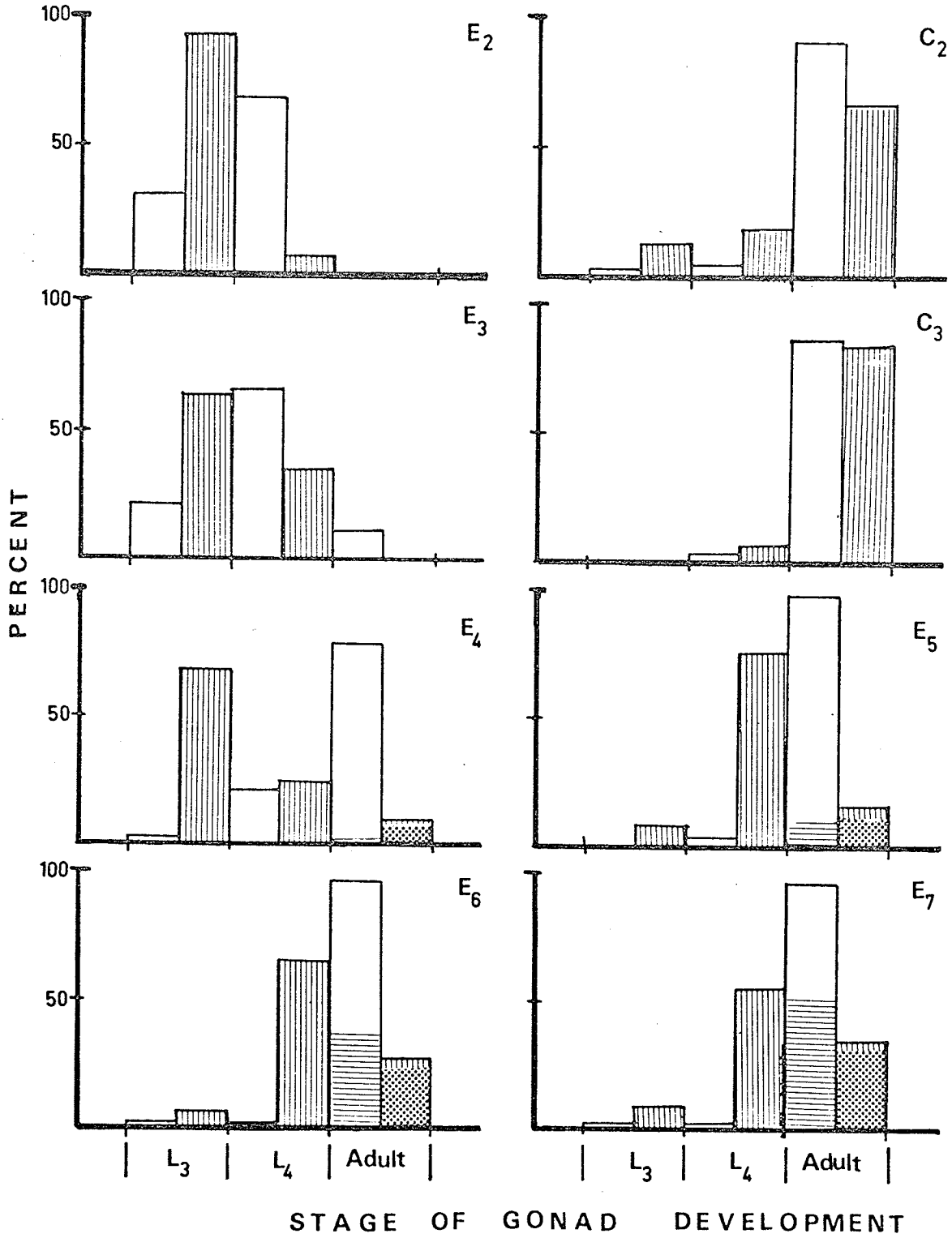
Figure 8. Percentage distribution of stages of gonad development in SPYE alone (E_2, E_3, E_4, E_5, E_6 and E_7 represent distribution in SPYE alone at days 4, 6, 8, 10, 12 and 14 respectively and C_2 and C_3 represent distribution at days 4 and 6 in SPYE + HLE. L_3 and L_4 represent the third and fourth juvenile stages.

 - normal male stages.

 - abnormal male stages.

 - normal female stages.

 - abnormal female stages.


100
50
E_2
C_2
E_3
C_3
E_4
E_5
E_6
E_7
PERCENT
L_3
L_4
Adult
STAGE OF GONAD DEVELOPMENT

Abbreviations in Figures 9 - 14.

b - Cuticular blister.

c - Cuticle.

ger - Germinal zone of gonad.

gro - growth zone of gonad.

L - developing larvae.

ps - presumptive sperm.

ov - developing eggs.

v - vulva.

Figure 9 - Testis of normal adult male at day 6 in SPYE + HLE (X 1000).

Figure 10. Testis of adult male at day 8 in SPYE alone (X 1000).

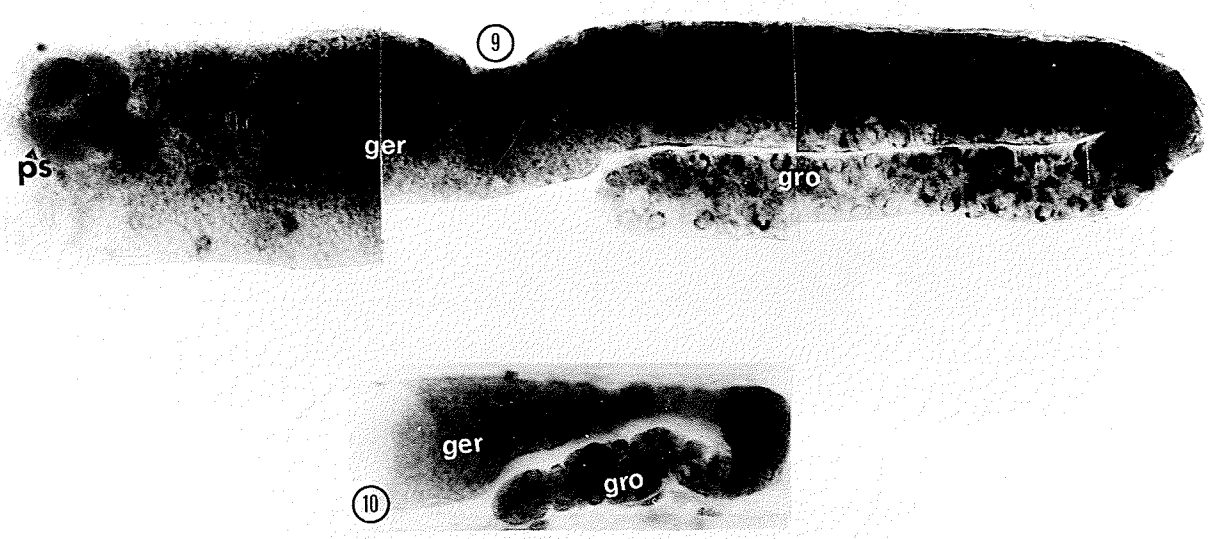

9
ps
ger
gro
ger
gro
10

Figure 11. Normal adult female gonad at day 6 in SPYE + HLE (X 200).

Figure 12. Abnormal female at day 10 in SPYE alone (X 200).

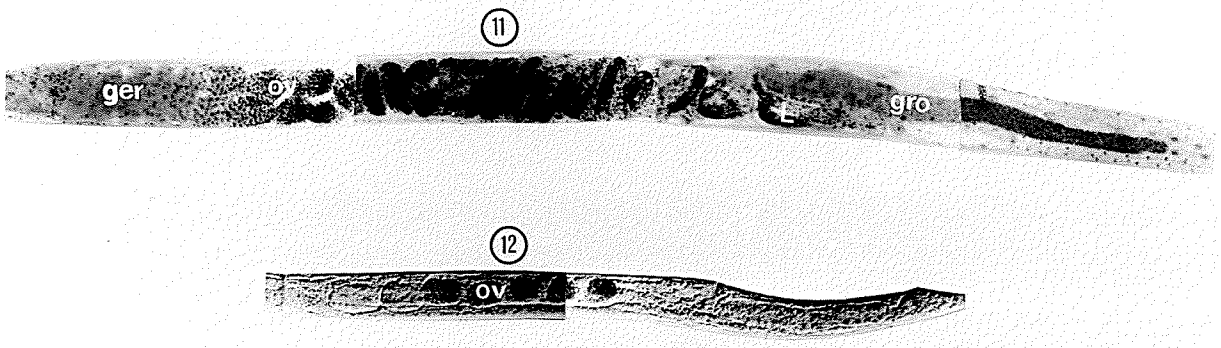

11
ger
ov
gro
12
ov

Figure 13. Abnormal female (14 days in SPYE alone) showing 2 vulva (X 1000).

Figure 14. Cuticular blister on an abnormal female at day 14 in SPYE alone (X 1000).

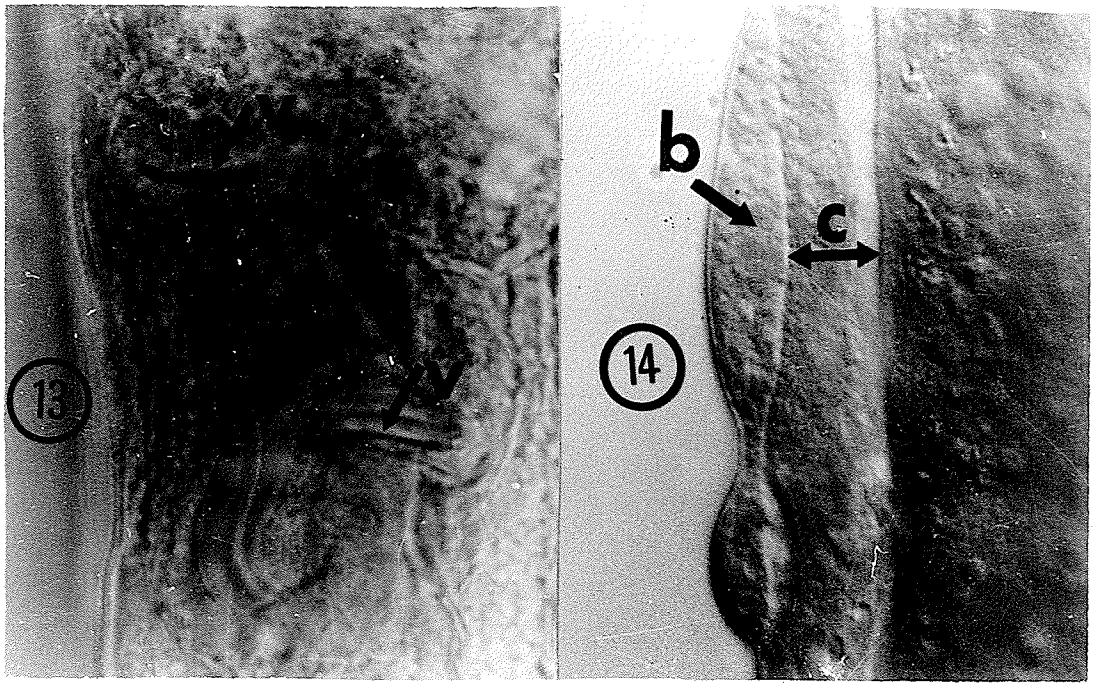

13
14
b
c

Figure 15. Effect of transfer from either salts + HLE or salts alone at various time intervals on growth. (e - salts + HLE, f - salts alone) - arrows indicate the time of transfer.

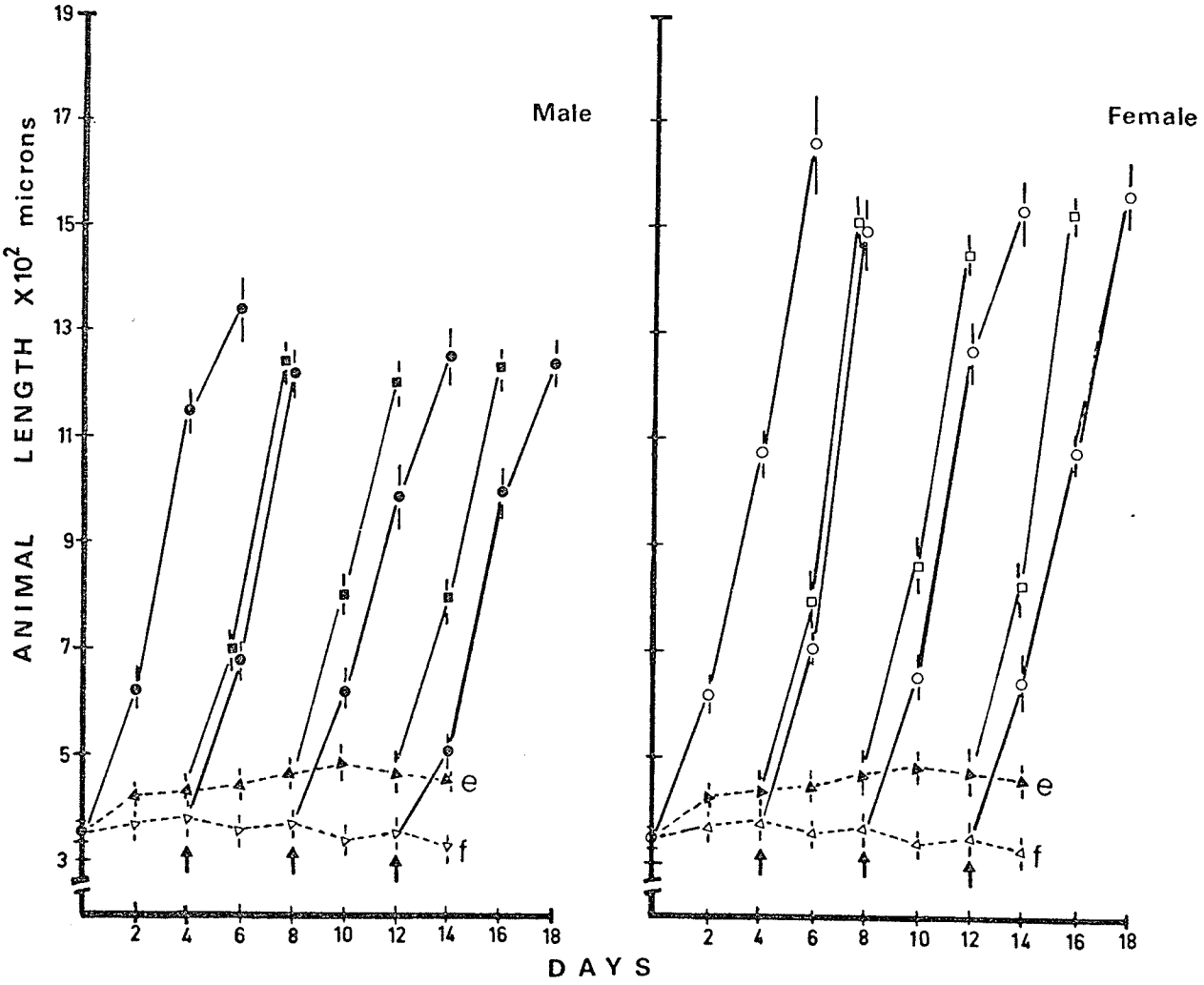

ANIMAL LENGTH X10² microns
19
17
15
13
11
9
7
5
3
Male
Female
e
f
2
4
6
8
10
12
14
16
18
DAYS

Figure 16. Effect of addition of HLE to SPYE maintained nematodes at various time intervals on growth (arrows indicate time of addition).

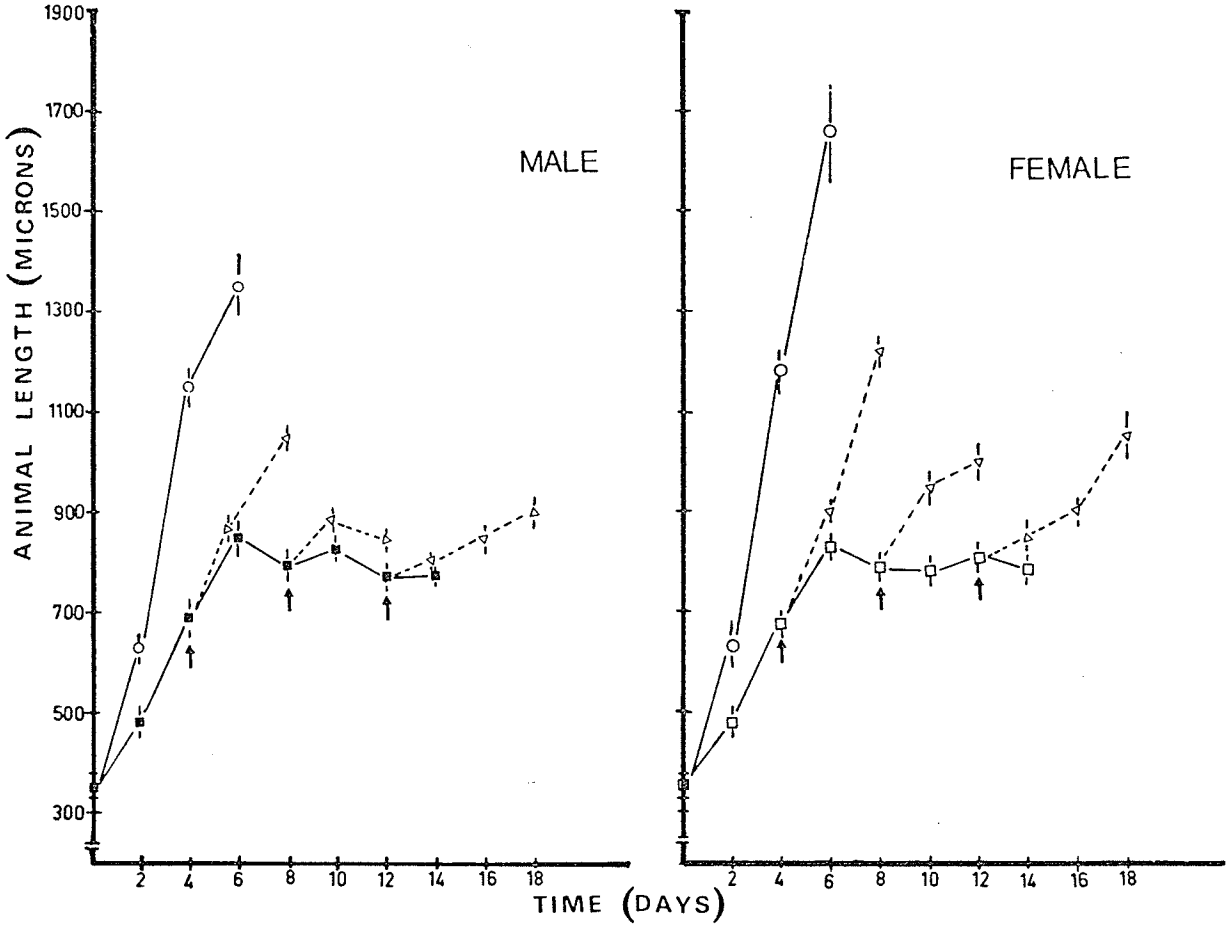

ANIMAL LENGTH (MICRONS)
1900
1700
1500
1300
1100
900
700
500
300
MALE
FEMALE
2
4
6
8
10
12
14
16
18
TIME (DAYS)

Figure 17. Survival curves of males transferred from SPYE alone to SPYE + HLE after 4, 8 and 12 days, (closed squares - immature forms after 4 days, open squares - immature forms after 8 and 12 days, closed triangles - SPYE alone males, closed circles - males after 4 days, and open circles - males after 8 and 12 days).

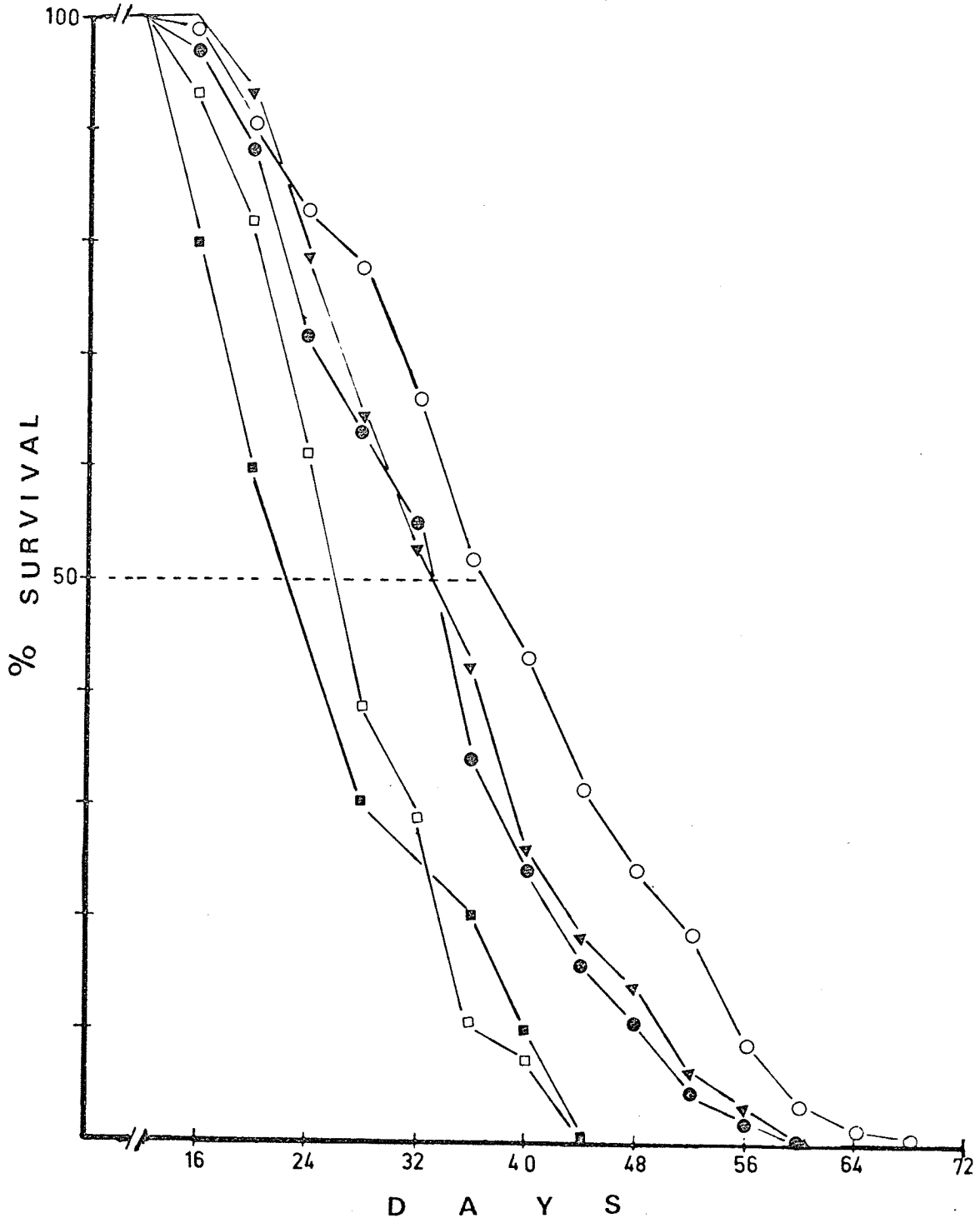
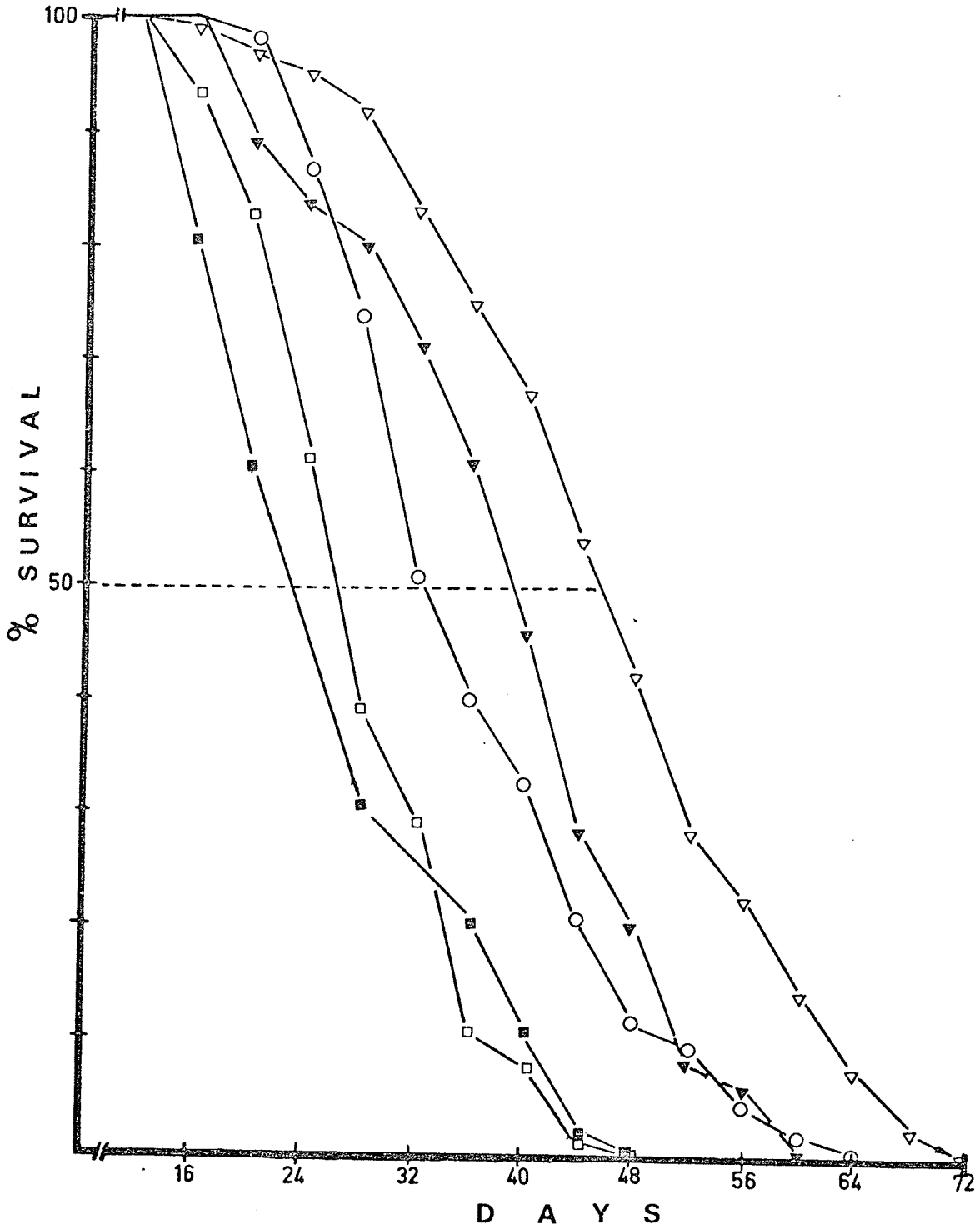

100
50
% SURVIVAL
16
24
32
40
48
56
64
72
D A Y S

Figure 18. Survival curves of females transferred from SPYE alone to SPYE + HLE after 4, 8 and 12 days, (closed squares - immature forms after 4 days, open squares - immature forms after 8 and 12 days, open circles - abnormals in SPYE alone, closed triangles - females after 4 days and open triangles - females after 8 and 12 days).


100
50
% SURVIVAL
16
24
32
40
48
56
64
72
D A Y S