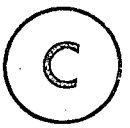
A DEVELOPMENTAL ANALYSIS OF BEHAVIOURAL MUTATIONS IN DROSOPHILA MELANOGASTER



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David T.L. Wong

B.Sc., Simon Fraser University, 1976

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Department of GENETICS

The University of British Columbia
2075 Wesbrook Place
Vancouver, Canada
V6T 1W5

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ABSTRACT

Two types of sex-linked recessive mutations in Drosophila melanogaster have been investigated in the present study. The first type includes 5 different mutations which exhibit a stress-sensitive (ses) phenotype. Flies of all five mutant stocks become paralyzed when their containers are lightly tapped; wild type flies are unaffected by the same treatment. The five mutations form three complementation groups (cistrons). Flies mosaic for mutant and non-mutant tissue were studied to determine the foci of their action in embryos by fate mapping. These studies suggest that the mutation ses D^2 has 6 foci in the presumptive nervous system of the blastoderm. Each focus corresponds to a site in the thoracic ganglion which controls the movement of one leg. The focus for ses E^1 mutation is rather diffuse and occupies a larger area in the thoracic nervous system of the blastoderm fate map. Focus mapping studies with the ses B^1 mutation were inconclusive because of the highly variable expressions of the adult behavioural phenotype in mosaic individuals. Developmental studies, involving temperature shifts from 22°C to 29°C (permissive to restrictive temperatures) revealed that the ses E^1 mutation has 2 temperature-sensitive periods (TSPs) for lethality during its development, one in the late 2nd larval instar stage and the other in the late pupal stage. In addition to developmental TSPs of ses B^2 at the embryonic, 1st larval instar and pre-pupal stage, temperature-shift studies also revealed a ts maternal-effect lethal for ses B^2.

The second type of mutation studied has a temperature-sensitive (ts) phenotype of adult death induced by shifting up to the restrictive temperature. The *add A*^{ts1} flies have normal behaviour and longevity at 22°C but die within 24 hours after shift-up to 29°C. In contrast, the *ses E*^{1} flies are less active and require 168 hours at 29°C to induce death. Both mutations studied have a 3rd larval instar-pupal TSP with *add A*^{ts1} and *ses E*^{1} also having an additional TSP at the embryonic and 1st larval instar-2nd larval instar stage respectively. Fate mapping studies suggest that the adult lethal phenotype of *add A*^{ts1} is caused by a lesoon in tissues derived from the mesodermal cells of the blastoderm, and for *ses E*^{1}, the lesion is in the neural cells.

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CHAPTER 1

GENERAL INTRODUCTION

The control of gene expression in bacteria and their viruses is now understood in detail. In addition to providing us with information on regulation of gene activity, these studies emphasize the vital role of each gene product in sustaining the life of an organism. A specific enzyme in a metabolic pathway can be removed by a mutation in the structural gene. This allows the determination of the cellular effects of the missing enzyme and often, the metabolic pathway. Such point mutations have also proven useful in the study of complex developmental pathways such as bacteriophage assembly (Edgar and Wood, 1966; Meezan and Wood, 1971), and the function of other macromolecular complexes including bacterial membranes (Silbert, 1975), ribosomes (Nomura and Morgan, 1977), flagella (Silverman and Simon, 1977) and chemotactic receptors (Alder, 1975). However, phenomena that only occur in multicellular organisms such as cell to cell interaction and communication, tissue differentiation, dosage compensation and co-ordinated behaviour are less well understood, and hence must be studied in them.

One approach to study co-ordinated behaviour in multicellular organisms is to use mutations which systematically disrupt neural development and function. The rationale behind this approach lies in the fact that both assembly and function of the nervous system is under the direction of genes. Proper performance of a given behaviour

requires the completion of several steps in a pathway. Each step in the behavioural pathway is a physiological process dependent on polypeptides coded by genes. Therefore, an alteration in the structure of a polypeptide would allow the studying of altered behaviour with respect to the malfunctioning of a component in the behavioural pathway. The use of single gene mutations thus allows sequence determination of the pathway by looking at these mutations one at a time.

The genetic approach to study behaviour via the disruption of neural function has several advantages over the otherwise surgical, chemical and developmental manipulations because (1) mutations are inheritable, (2) their effects are very specific, (3) genetic manipulations are minimal when compared to other approaches and (4) statistical study of behaviour is possible through the use of a clone of mutants.

To study the behaviour of multicellular organisms, it is necessary to find an experimental animal possessing a complex nervous system as well as exhibiting a rich repertoire of endogenous patterned behaviour. Insects are ideal candidates for this purpose of studying the function of the nervous system. Among them, Drosophila is chosen for extensive behavioural study because of its complex nervous system (approximately 10^5 neurons vs. 10^{11}-10^{12} neurons in vertebrates) and its well studied behavioural patterns. For example, mating behaviour is often characterized by the male following the female, tapping the female's abdomen, extending and vibrating a wing in a specific grequency to produce a species-specific courtship song (Hotta and Benzer, 1976).

The stereotyped behaviour also includes a positive optomotor response demonstrated by flies quickly adjusting their movement to changes in direction of a turning cylinder lined with alternate black and white stripes (Heisenberg and Gotz, 1975). Moreover, flight behaviour in Drosophila is a complicated process involving resonating the thoracic box by alternately stretching and relaxing different muscles (Levine and Tracey, 1973). In addition, Drosophila exhibit negative geotactic and positive phototactic behaviour (McEwen, 1918; Benzer, 1967).

The well known genetics in Drosophila which facilitate the induction, isolation and genetic characterization of mutations is another reason why it is chosen for behavioural studies. Potent chemical mutagens (for example, ethyl methanesulfonate (EMS)) which induce point mutations have allowed the detection and recovery of a wide array of mutations which ahve provided an enormous amount of information on processes such as biosynthetic steps (Falk and Nash, 1974), macromolecular assembly (Homyk, unpublished) and, more recently, behaviour. Behavioural mutants unable to perform one or all of the courtship steps have been isolated and characterized successfully (Hotta and Benzer, 1976). Mutants that have abnormal geotactic and phototactic responses have also been studied (McEwen, 1918; Benzer, 1967). In the latter studies, many non-phototactic mutants recovered were characterizable by abnormal variations in their electroretinograms (ERGs). An electroretinogram is an extracellular recording of a light induced electrical response from the eye. In fact, mutations have been identified that specifically

cause abnormalities in laminal cells and retinula cells that are indicated by an abnormal optomotor response and ERG respectively (Pak and Grabozski, 1978). Recent work has also focused on the flight behaviour of Drosophila via the use of several flightless mutants (Homyk, unpublished). Thus, the use of these behavioural mutations, its genetic amendability, together with the new use of neurophysiology in Drosophila, such as intracellular recording techniques (Ikeda and Kaplan, 1970a), outweigh the disadvantages of its small size. In fact, such techniques have helped to reveal the complex neural-motor circuit (Kaplan and Trout, 1969; Ikeda and Kaplan, 1970a; Ikeda and Kaplan, 1974; Jan and Jan, 1978) in Drosophila.

Behavioural abnormalities could be caused by mutations affecting the assembly of the nervous system. Hence, the development of the nervous system must be studied. Drosophila has been a favourite organism for studying the genetic control of developmental processes in a multicellular eukaryote. Its developmental process (Zalokar, Audit and Erk, 1978) as well as cell differentiation and determination (Lewis, 1978) have been studied in detail. It is chosen for developmental studies because of its complex life cycle involving metamorphosis from egg to larva to pupa to adult in a relatively short time (12 days at 22°C). Its development is most intriguing to both developmental biologists and geneticists owing to the vast differences in the anatomical structures between the adult and the larva as well as the transformation process from the larva to an adult with wings, legs and compound eyes which

initially were never presented.

Among the many tools used in the studying of Drosophila development, temperature-sensitive (ts) mutations have proven to be a valuable asset. They allow expression of a mutant phenotype only at the restrictive temperature while being normal at the permissive temperature (Suzuki, 1970). Temperature-sensitivity in micro-organisms is a consequence of a single amino acid substitution in a polypeptide which alters the biological activity of a protein at different temperatures (Jockush, 1966) rather than an effect on the actual process of transcription or translation. In Drosophila melanogaster, ts lethal mutants similar to those of micro-organisms have been recovered (Suzuki et al., 1967; Baillie et al., 1968). These mutants have temperature-sensitive periods (TSPs) which are often readily defined during development by appropriate shifts of cultures from permissive to restrictive temperatures. The ultility of ts mutations is manifold: they obviate the need to engineer special balanced stocks of lethals, they allow recovery of mutants that could not be detected otherwise (such as dominant lethals, Suzuki and Procunier, 1969) and most important of all, they permit the delineation of TSPs to the time of gene action during development.

One question often asked concerning behavioural mutations is where in the animal the mutation is exerting its effects. Answers to this question are often complicated by problems evident in the following example. The defective tissue in a non-phototactic mutant may be in parts of the nervous system that are not directly relevant to the visual

physiology, but to a mere motor defect. Hence, it is necessary to investigate which component in a behavioural pathway the mutation is affecting. In Drosophila, genetic mosaics have been used extensively to provide answers to these questions (Hotta and Benzer, 1970; 1972; Ikeda and Kaplan, 1970b; Suzuki et al., 1971; Grigliatti et al., 1972; Kankel and Hall, 1976). Genetic mosaics are individuals composed of genotypically distinct types of tissue. The generation of mosaics often required manipulations of the fly's genotype through the use of chromosome mechanics. The more sophisticated genetic tools available to Drosophila has allowed it to become one of the most extensively developed mosaic systems of all organisms (for mosaic systems in other organisms, see review by Pak and Pinto, 1976). Other uses of mosaics in behavioural analysis have involved questions on specific interaction between normal and mutant tissues. Thus, the problem of whether the phenotype of a genotypically normal tissue in a mosaic is affected by a circulating substance produced by a mutant tissue elsewhere can be solved.

One powerful technique of analysing genetic mosaics is to extrapolate the site of the mutation back to the embryonic cells. This method of determining the embryonic tissue within which behavioural mutants act has been described in detail by Hotta and Benzer (1972). It is based on the assumption that in a fertilized Drosophila egg, the polar orientation of the spindle in the first mitotic division is randomly positioned within a aphere (Fig. 1A) (Parks, 1936). Following this, nine synchronous nuclear divisions occur without cell division.

The nuclear derivatives of the two nuclei formed after the first division remain clustered with little mixing (Fig. 1B). Following these nine divisions, the nuclei migrate to the surface of the blastula where they divide three more times after which cell membranes form around them (Fig. 1C). Once the blastoderm is formed, the cortical position occupied by a cell determines its developmental fate; the anterior region of the blastoderm gives rise to anterior structures of the adult fly (Hathaway and Selman, 1961; Chan and Gehring, 1971). It is obvious that for any two sites on the blastoderm, the further apart they are, the more likely a mosaic boundary would lie between them (Fig. 1D). Hence, in a population of mosaics, the frequency with which any two adult landmarks are of different phenotypes is related to their proximity in the blasoderm.

A map of the blastoderm position of structures on the external surface of adults can be constructed (Fig. 2) from the distribution of mutant and wild type tissues in a population of mosaics. The frequency with which any two surface landmarks are phenotypically different, is said to be the "map distance" on an embryonic fate map. This is analogous to mapping two linked genes based on the frequencies of crossing over between them. A fate map differs in that it allows a two dimensional construction based on the triangulation of three different structures (Fig. 3).

The embryonic foci of behavioural mutants can also be determined by triangulating a behavioural phenotype relative to several different external cuticular markers. This is done by measuring the frequency

Figure 1. Formation of a mosaic fly by loss of one X chromosome. Inital egg contains a ring-X chromosome plus a rod-X chromosome carrying recessive genes for marking the body surface as well as the behaviour to be studied. (A) Loss of ring-X at the first division results in a rod-X/0 nucleus (open circle) and a ring-X/rod-X nucleus (solid circle). (B) The nuclei devide in cluster without mixing then migrate to the egg cortex (C) where cell membranes form to produce a one cell thick blastoder. (D) Surface view of the blastoderm with the mosaic boundary seperating the two cell types.
(from Hotta and Benzer, 1972)

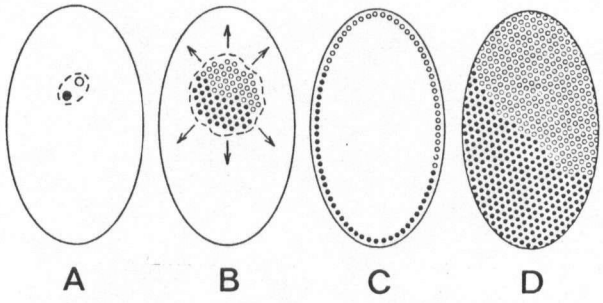
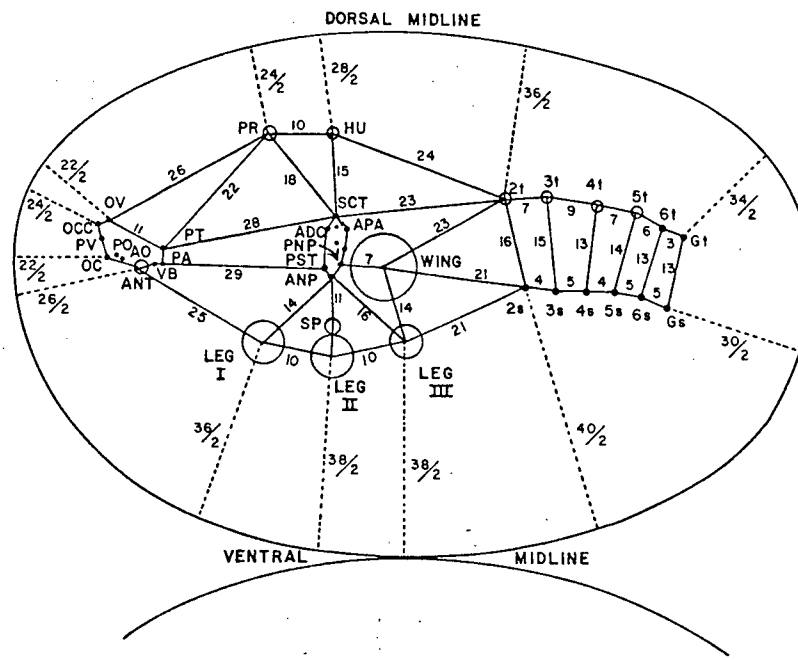

A
B
C
D

Figure 2. Right half of the fate map of Drosophila melanogaster for adult external body parts, constructed by mosaic mapping. A mirror image of the map corresponds to the left half. Dotted lines indicate distances to the nearest midline, as obtained by halving the distance measured between two homologous sites on each side. Distances are indicated in sturts. One sturt is defined as a distance such that the two external body parts are of different genotypes in 1% of the mosaics. The size of the circle is proportional to the frequency with which the structure is itself split by the mosaic boundary.
(from Hotta and Benzer, 1972)


DORSAL MIDLINE
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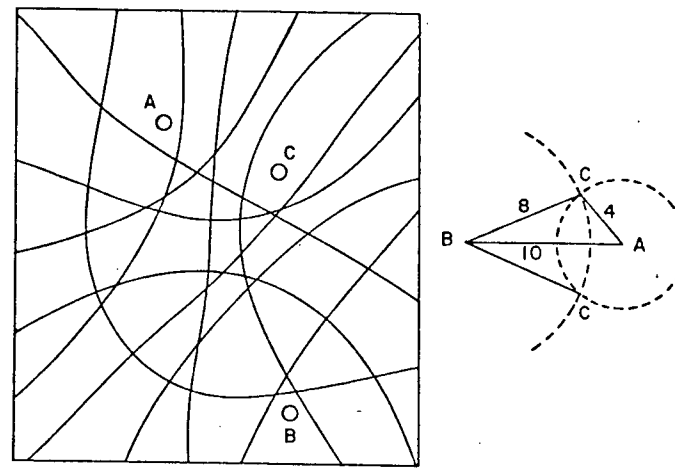
1t
2t
3t
4t
5t
6t
Gt
1s
2s
3s
4s
5s
6s
Gs
24/2
28/2
36/2
22/2
34/2
26/2
30/2
36/2
38/2
38/2
40/2

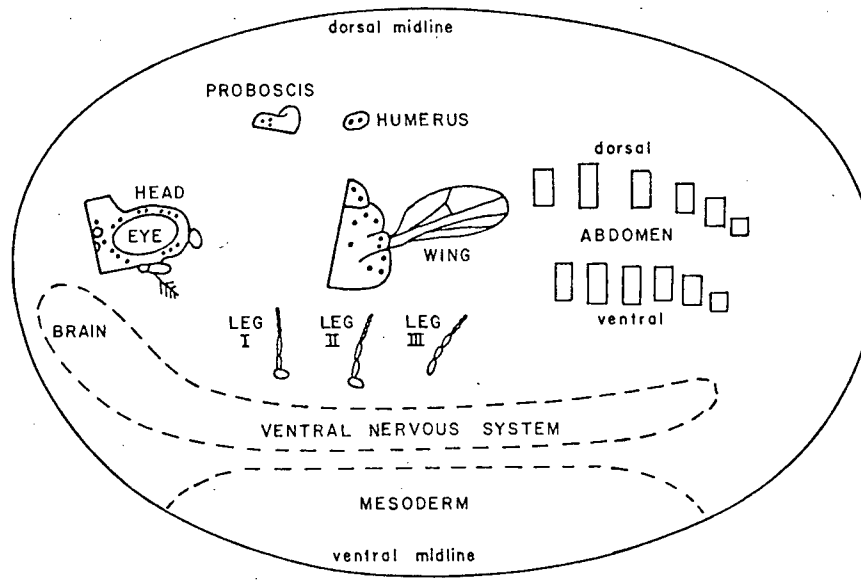
Figure 3. Principle of mosaic fate mapping. Left: Mosaic boundary lines fall in random orientation on the blastoderm. The farther apart two sites are, the more likely it is that the boundary will fall between them, and hence the structures derived from these sites will be of different genotype. The distance between two sites on the fate map is indicated by the frequency with which two cuticle landmarks are of different phenotypes. Right: Location of a third site by triangulation. Sites A and B are 10 sturts apart. If C is 4 sturts from A and 8 sturts from B, then C can be located. A choice between the two possibilities requires mapping with respect to additional sites. (from Hotta and Benzer, 1972).


A
C
B
C
8
4
B
10
A
C

with which mutant behaviour is found when an external marker is wild type or vice versa. Using a second set of external landmarks, the distance between the "behavioural focus" and the second landmark can be determined. Using a number of these surface landmarks, the localization of the "behavioural focus" responsible for the mutant phenotype is therefore possible. Embryological studies have delineated the neural and muscular tissues in the embryo (Poulson, 1965) and fate mapping of mutations with known neurological defects finds their mapped embryonic foci congruent with the cytological picture (Fig. 4).

Thus, the genetic and developmental tools developed in Drosophila provide a means of dissecting the components of behaviour. This report involves the use of new mutations and focus mapping in an attempt to find out where, when and how specific genes control neurobiological phenomena. This includes how genes affect the assembly and function of the nervous system throughout the life cycle of the organism. Also, an attempt will be made to pinpoint the focus of its action and determine whether it is affecting the muscles or the nervous system. Moreover, the time and duration of its action will be investigated.

Figure 4. Right half of the fate map of Drosophila melanogaster for adult external body landmarks. Dotted lines, according to embryological studies (Poulson, 1965), indicate areas of presumptive nervous system and mesoderm. (from Hotta and Benzer, 1972)


dorsal midline
PROBOSCIS
HUMERUS
dorsal
HEAD
EYE
WING
ABDOMEN
ventral
BRAIN
LEG I
LEG II
LEG III
VENTRAL NERVOUS SYSTEM
MESODERM
ventral midline

CHAPTER 2

STRESS-SENSITIVE MUTATIONS

I. Introduction

A number of mutations conferring sensitivity to mechanical shock have been reported (Judd, Shen and Kaufman, 1972; Grigliatti et al., 1973; Feitelson and Hall, 1975). Such flies are extremely sensitive to stress. A light tap of the vial containing these flies is usually sufficient to induce immediate paralysis.

Early studies on stress-induced paralysis in the American cockroach, Periplaneta americana (Cook, 1967) revealed that shock induces production or release of a neuroactive substance, Factor S, which causes an initial rise in nervous activity followed by a blockade in the nervous system. The behavioural response of the insect to stress parallels the above physiological observations; an initial hyperactivity followed by paralysis (Cook and Holt, 1974). The initial rise in nervous activity is the familiar physiological phenomenon of facilitation. Facilitation is an enhanced post-synaptic response caused by repeated pre-synaptic activity. Since facilitation is an insect's normal response to stress, an abnormal facilitation pattern might be expected among some stress-sensitive mutants. Indeed, a bang-sensitive mutant, bas, exhibits an abnormal facilitation pattern at the neuromuscular junction (Jan and Jan, 1978). This facilitation pattern develops much faster and decays much more slowly than normal. Fate mapping analysis of

bas indicated that its primary focus is localized to the prothoracic ganglion (Feitelson and Hall, 1975). Thus, taken together, data for bas indicate that the mutant defect is most likely pre-synaptic in origin. The stress-sensitivity phenotype may characterized a class of defects that will be useful for studying neuromuscular junctions.

In the present study, the properties of recessive stress-sensitive mutations in three sex-linked genes (ses B, ses D, ses E) are investigated. The mutants studied were all recovered by Homyk and Sheppard (1977) and Homyk, Szidonya and Suzuki (1980). They were analyzed for their embryonic foci by fate mapping, their response to different neurotropic drugs and, in the case of temperature-sensitive alleles, their temperature-sensitive periods were delineated.

II. Materials and Methods

Except where specified, all cultures were grown on standard cornmeal-yeast-dextrose *Drosophila* medium at 22°C ± 1°C. The isolation procedures, mapping and behavioural properties of *ses* B^1, *ses* B^2, *ses* D^1, *ses* D^2 and *ses* E^1 have been reported (Homyk and Sheppard, 1977; Homyk, Szidonya and Suzuki, 1980). The map positions of the genes are: *ses* B, 1-33.0; *ses* D, 1-20.0; *ses* E, 1-27.7. The behavioural characteristics of the mutants and Oregon-R wild type flies used in this study are summarized in Table 1.

Developmental Studies

The mutations *ses* B^1 and *ses* E^1 were found to be heat-sensitive lethals, that is, while surviving at 22°C, these flies die at 29°C. Temperature-sensitive periods (TSPs) for heat-sensitive lethality are delineated by the earliest shift down which reduces viability and the earliest shift up yielding significant levels of survival (Suzuki, 1970). All shift experiments were performed between 22°C (permissive) and 29°C (restrictive). The developmental stage present in each culture at the time of shift was determined by inspecting developing flies at that time. Larval instars were determined by the morphology of their mout parts and anterior spiracles (Bodenstrin, 1950).

Flies from which eggs were to be collected were placed in empty bottles whose mouths were covered with a watch glass. The watch glass carried a strip of agar (1% agar in boiling water, 2% ethanol

Table 1

Behavioural characteristics of wild type Oregon-R and mutant flies used in this study.

Mutants	Total number of flies observed	Behaviour before tapping of vial	Behaviour after 1 tap to make flies fall to the bottom of the vial	Behaviour after 5 - 10 sec. of tapping
ses D^1	20	active	Climb up the walls of the vial quickly	Flies climb up slowly and occasionally fall back. Require 90 secs. for full recovery. 4 flies paralyzed, require 1-2 min. to recover.
ses D^2	"	"	"	"
ses E^1	"	"	Climb up the walls of the vial quickly	All flies begin to climb up the walls of the vials after they were paralyzed for 5 to 10 secs.
ses B^1	"	inactive	Climb up the walls of the vial slowly	After tumbling and falling on their backs for 30 secs., 10 flies recovered. The other 10 remained paralyzed and required more than 3 min. to recover.
ses B^2	"	moderately active	Climb up the walls of the vial slowly	After tumbling and falling on their backs for 30 secs., 16 flies recovered. The other 4 remained paralyzed and required more than 3 min. to recover.
Ore-R	"	active	Climb up the walls of the vial immediately and very quickly	None paralyzed. Flies climb up the walls of the vial immediately and very quickly.

and 1% acetic acid) covered with yeast paste (yeast and tap water mixed). Since females can retain fertilized eggs before deposition, the first two-hour egg collection was discarded. The second two-hour collection was saved and the eggs removed from the agar strip by washing. These developmentlaly synchronized eggs were collected on a fine wire mesh and subsequently transferred to a 20% sucrose solution which allows the debris (yeast paste and agar) to sink to the bottom of the beaker while the eggs float on top of the solution. The eggs were then re-collected on the wire mesh and washed thoroughly with distilled water. The final washing of the eggs is essential to ensure that all traces of sucrose are completely removed to prevent mould growth. After the washing, the eggs were counted and laid on strips of heat-sterilized moistened blotter paper which were then placed in fresh vials containing Drosophila medium. Forty-eight hours later, the paper strips were removed and the number of unhatched eggs noted.

Three sets of cultures were established for shift experiments. Following egg collection, one set was left at 22°C and another was immediately transferred to 29°C. At successive intervals of 12 hours, cultures were shifted up or down accordingly. 24-hour heat pulses were also administered to the third set of cultures at different times of development. All cultures were scored for the number of pupae formed and the number of adults eclosed.

Genetic Mosaic Studies

Since all of the mutations were located away from the tip

of the X chromosome, the distally located external cuticular markers, yellow and white were readily combined with them by crossing over. Genetic mosaics were generated through the use of the somatically unstable ring-X chromosome, $In(1)w^{vC}$ (Hinton, 1955). Females carrying the $In(1)w^{vC}$ chromosome were mated to males carrying the specific behavioural mutations (ses) linked to the cuticular markers yellow and white. These males are y w ses/Y. By crossing $In(1)w^{vC}$/y w spl females to y w ses/Y males, $In(1)w^{vC}$/y w ses female zygotes can be recovered. Loss of the $In(1)w^{vC}$ ring-X chromosome in the early cleavage stage (Garcia-Bellido and Merriam, 1969) of $In(1)w^{vC}$/y w ses female zygote results in patches of y w ses/O male tissue in the adult fly. Since this tissue is hemizygous, the mutant phenotype can be expressed provided the gene is active in those cells. The location and extent of mutant tissue are determined externally by expression of the yellow and white phenotype on a background of wild type tissue. The areas of mutant tissue were recorded for each mosaic and then its behaviour at 29°C was noted before and after each shock treatment. If the mosaic fly became completely paralyzed after a shock, the time for its recovery was recorded. Partial paralysis was also recorded in those cases where the fly dragged itself along using one or more pairs of legs. In this manner, the mosaics were examined on a daily basis for at least two weeks.

Neurophysiological Studies

In order to test whether synaptic functions are involved in the ses mutant defects, the mutants were exposed to varying con-

centrations of a variety of drugs known to be psychotropic and neurotropic in mammals. The number of drugs used, their larval lethal dose and their known physiological effects are listed in Table 2.

Each mutant strain was subjected to at least 5 different concentrations of each drug. The concentrations were $\frac{1}{4}$, $\frac{1}{2}$, 1, 2 and 4 times the larval lethal dose as reported by Howard, Merriam and Meshul (1975) or determined in the present study. Where extra sensitivity or extra resistance was noted, the experiments were repeated with lower ($\frac{1}{8}$ times the larval lethal dose) or higher (8 times the larval lethal dose) concentrations, respectively.

5 ml. of each drug of appropriate concentrations (see above) were added to 1.4 gram of instant Drosophila medium (Boreal) to make 6 ml. of medium. Where possible, distilled water at room temperature was used to dissolve the drug. In order to prevent any heat inactivation when the solubility of the drug at room temperature presented a problem, the water temperature was raised just enough to dissolve the drug. To prevent bacterial growth, two antibiotics, streptomycin and tetracycline (10 mg./litre) were added to the distilled water while mould growth was inhibited with tegosept (100 gm./litre ethanol).

Newly deposited eggs were collected and counted on strips of moistened blotter paper as outlined above and then placed into vials containing drugs of different concentrations. These were subsequently scored for the number of pupae formed and the number of adults eclosed both at 22°C and 29°C.

Table 2

List of drugs, their putative action and the Drosophila larval LD_{50}. Taken in part from Howard, Merriam and Meshul, 1975.

DRUG	Putative action	Larval lethal dose, mg/ml of instant Dros. medium (Boreal)
Carbamylcholine chloride	Cholinonimetic	3
Pilocarpine nitrate	"	0.8
Succinylcholine chloride	"	2.5
Nicotine	"	.06
Atropine sulfate	Cholinergic blocker	6
Hexamethonium bromide	" "	2
d-tubocurare	" "	4
Eserine sulfate	Inhibits acetylcholinesterase	.025
Reserpine	Reduces transmitter storage	.2
Nialamide	Inhibits monoamine oxidase	1
Iproniazid	" " "	1
Caffeine	Inhibits cyclic nucleotide phosphodiesterase	0.5
Theophylline	" " " "	0.5
Strychnine sulfate	Glycine antagonist	2
Picrotoxin	GABA antagonist	.01
Allylglycine	Convulsant	5
Amino oxyacetic acid	Inhibits GABA degradation	.8
Methionine sulfoximine	Convulsant	.5
Sodium barbital	Nerve, muscle depressant	.5
Urethane	Muscle depressant	1
Dopamine	Neurotransmitter	5
dl-Norepinephrine	"	7
Tyramine	False transmitter	3
dl-Octopamine	" "	10
Histamine	Neurotransmitter	7.5
GABA	"	25
Glutamic acid	"	25
Catechol	Adrenergic blocker	4
Yohimbine	" "	2

III. Results

ses B

Characterization of TSPs: The temperature-sensitive phenotype of the ses B mutation enabled analysis of gene activity by temperature-shift experiments. The ses B^2 mutant exhibit a ts maternal-effect. A ses B^2 stock maintained in the male line as ses B^2/Y males X y f:=/Y attached-X females at 29°C yields a 1:2 ratio of males to females in each generation whereas the stock in which homozygous ses B^2 females have been kept at 29°C yields no adult progeny at all. This ts maternal-effect was further investigated by mating ses B^2 homozygous females to Oregon-R males at 29°C. Only 4 males were recovered with 291 females survived (Table 3). In addition, males from the cross ses B^2/Y X y f:=/Y attached-X females and ses B^2/Y X ses B^2/ses B^2 females survive well at 22°C (90% and 73% viable progeny respectively). This ts maternal-effect was also investigated ny shift studies. Adult females from the homozygous stock were shifted from 22°C to 29°C and eggs collected and counted while their subsequent development was scored. Females, kept at 29°C for more than 3 days, laid eggs unable to give rise to adults. Before the 3rd day, about 10% of the eggs produced adults. However, eggs collected from females which had been held at 29°C for more than 3 days and shifted down immediately to 22°C yielded 50% adult eclosion. These females, which had been kept at 29°C for some time, were then transferred back to 22°C and in subsequent collections, eggs were monitored

Table 3

Maternal-effects of ses B^2. The progeny were reared at the same temperature the cross was made.

Cross	ses B^2 females X Ore-R males at 29°C		ses B^2 females X Ore-R males at 22°C	
eggs collected	930		750	
pupae formed	327		726	
Progeny recovered	males	females	males	females
	4	291	296	403

Cross	ses B^2/Y males X y f:=/Y females at 29°C		ses B^2/Y males X y f:=/Y females at 22°C	
Progeny recovered	males	females	males	females
	135	236	186	205

Cross	ses B^2/Y males X ses B^2/ses B^2 females at 29°C		ses B^2/Y males X ses B^2/ses B^2 females at 22°C	
Progeny recovered	males	females	males	females
	0	0	152	175

for further development. More than 4 days at 22°C were required before survival reached the normal value (more than 80%) of adult eclosion (Table 4). Eggs of control Oregon-R females held at 29°C for any period of time always had more than 75% adult eclosion.

Shift studies also indicated that ses B^2 has a 3rd instar-pupal TSP. A shift-up after 240 hours at 22°C gives a significant level of survivors, thus marking the end of the TSP. However, the beginning of the TSP could not be well defined since the duration of the 3rd instar-larval period could not be determined. When raised at 29°C, a large fraction of the 3rd instar larvae can survive without pupating for as long as three weeks before they begin to die. Nevertheless, shift-down experiments indicate that after 192 hours at 29°C (the end of 2nd instar-larval period is 144 hours at 29°C) the level of survival to adulthood is reduced (Fig. 5, Table 5). This may reflect either the beginning of the TSP or alternatively the time after which survival at 29°C is not possible. Therefore, the TSP is estimated to be between the 3rd instar larva and beginning of pupation.

It should be noted that in successive shift-up experiments, most of the larvae were able to pupate but failed to eclose as adults. In contrast, in the shift-down experiments, a large number of the larvae shifted during later stages of development were unable to pupate (Table 5). When ses B^2 embryos or 1st instar larvae were subjected to 29°C heat shocks, their ability to pupate was greatly reduced. Therefore, it seems that ses B^2 could have another TSP during the embryonic and

Table 4

Temperature-sensitive maternal-effect of ses B^2. ses B^2/ses B^2 females was crossed to ses B^2/Y males.

females lived at 22°C for ____ days	females lived at 29°C for ____ days	eggs laid at ____ °C	for ___ days	eggs developed at ____°C	Number of eggs collected	number of pupae	pupae/eggs %	number of adults	adults/eggs %
2		22	1	22	279	258	92	250	90
3		22	1	29	205	135	66	21	10
	1	29	1	29	275	209	76	37	13
	2	29	1	29	100	68	68	10	10
	3	29	1	29	124	59	48	9	7
	5	29	2	29	142	59	42	8	5
	6	29	1	29	70	21	30	0	0
	7	29	1	29	134	54	40	3	2
	8	29	1	29	100	22	22	1	1
	9	29	1	29	62	12	19	0	0
	10	29	1	22	52	27	52	26	50
2		22	2	22	200	127	64	124	62
3		22	1	22	52	33	63	33	63
4		22	1	22	112	100	89	98	88
5		22	1	22	77	56	73	55	71

Figure 5. Percentage of ses B^2 adults eclosed from hatched eggs after, ▲, shift-ups and ▼, shift-downs administered at different times during development. The developmental stage 22°C at the time of shift is shown at the bottom. The duration of 3rd instar stage cannot be determined due to non-pupating characteristics of this mutant at 29°C, hence, the curve for the shift-down cannot be drawn.

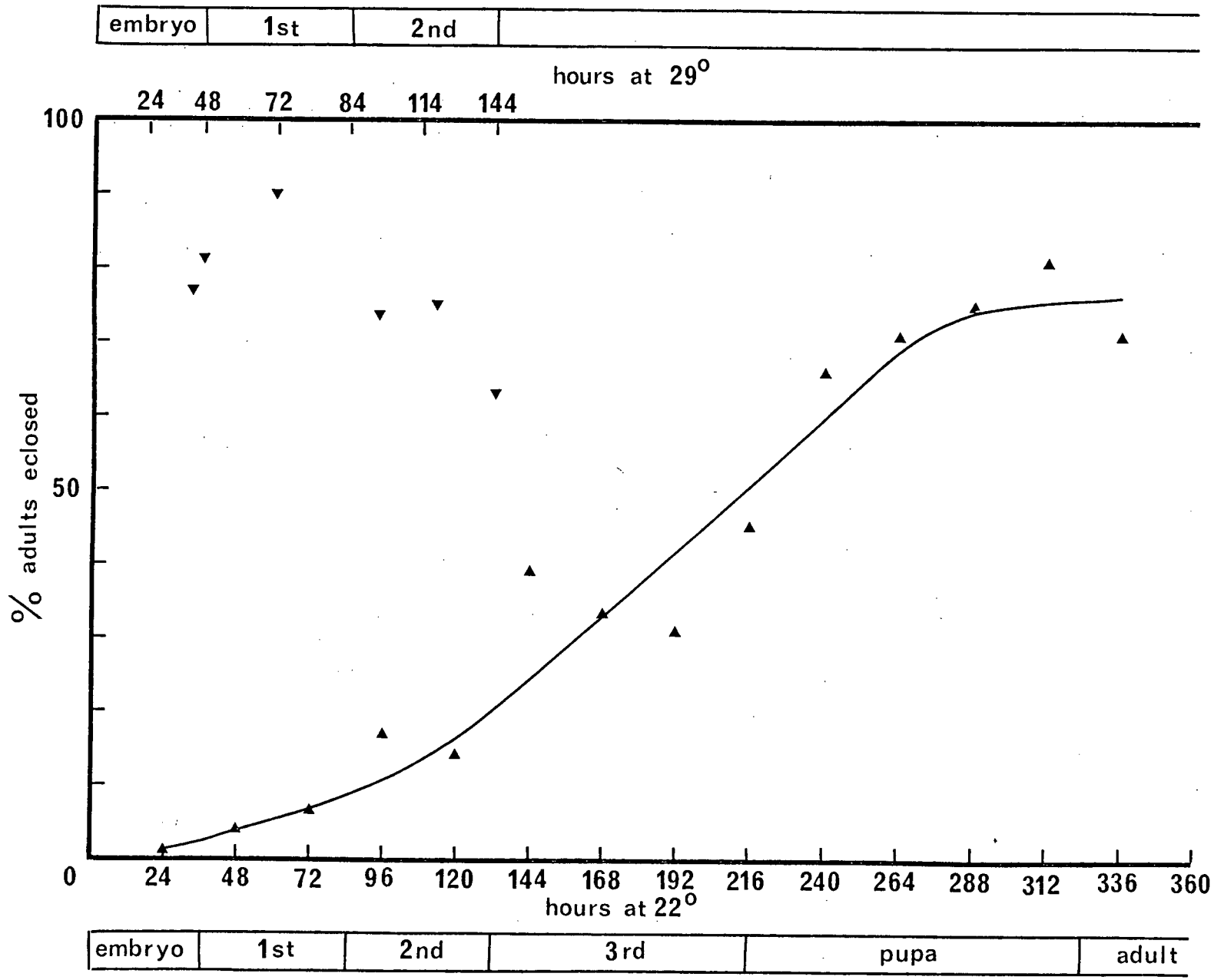

embryo
1st
2nd
hours at 29°
24
48
72
84
114
144
100
50
0
% adults eclosed
24
48
72
96
120
144
168
192
216
240
264
288
312
336
360
hours at 22°
embryo
1st
2nd
3rd
pupa
adult

Table 5

Temperature-shift studies of *ses* B^2. Eggs were collected at 0 hour. For shift-up experiments, eggs were collected at 22°C over a 2-hour period and left at 22°C until subsequent shifts. For shift-down experiments, eggs were collected at 22°C over a 2-hour period, immediately transferred to 29°C and left at that temperature until subsequent shift-downs.

hours	number of eggs	hatched eggs	number of pupae	number of adults	pupae / larvae %	adults / pupae %	adults / larvae %
			SHIFT-UP EXPERIMENTS				
0	egg collection						
24	78	74	54	1	73	2	1
48	79	73	55	3	75	6	4
72	74	70	59	5	84	8	7
96	78	76	64	13	84	20	17
120	77	69	62	10	90	16	14
144	76	70	65	27	93	42	39
168	77	72	67	24	93	36	33
192	80	74	63	23	98	37	31
216	79	73	60	33	82	55	45
240	77	71	59	47	83	80	66
264	74	68	65	48	96	74	71
288	77	67	61	50	91	82	75
312	77	72	64	58	89	91	81
336	38	35	35	25	100	71	71
			SHIFT-DOWN EXPERIMENTS				
0	egg collection						
45	79	74	58	57	78	98	77
48	70	78	66	63	85	95	81
72	74	71	65	64	92	98	90
96	77	72	58	53	79	91	74
120	77	71	59	53	83	90	75
144	76	70	61	44	87	72	63
168	75	74	65	53	88	82	72
192	75	71	57	35	80	61	49
216	75	69	52	28	75	54	41
240	80	79	39	16	49	41	20
264	78	73	31	12	42	39	16
288	80	79	48	13	61	27	16
312	79	75	43	11	57	26	15
336	76	73	24	1	33	4	1

1st instar stages of develcpment.

Genetic Mosaic Studies: ses B^1 is extremely sensitive to stress and usually requires more than 3 minutes to recover after paralysis. 271 mosaics were collected and the behaviour of each were examined in several trials. Mutant or normal behaviour could be unequivocally assigned to only 109 mosaics. Of the 109 mosaics, 50 were classified as mutant and 59 were classified as normal. The remaining 162 mosaics were left unclassified due to the inconsistency of their behaviour in seperate trials. Thus on one day they might be very bang-sensitive while on another day, they would be completely normal. The inability to assign a behavioural phenotype to these mosaics prevents the application of a conventional fate mapping analysis. However, the results suggest that the behavioural phenotype is most likely correlate to the phenotype of the cuticle markers in the head and thorax, and to a lesser extent, the phenotype of the abdomen (Table 6).

Drug Studies: Only those strains in which drug treatment resulted in a significant alteration of the percent pupariation or adult eclosion as compared to the wild type strain, Oregon-R, will be discussed. The results for the drugs are shown in Table 7. All responses to different concentration of various drugs which are not shown in the table did not differ from the Oregon-R wild type (Appendix). The results can be summarized as follows:-

A mutant is more sensitive or less resistant to a particular drug if its larvae have a lower viability than Oregon-R at the same

Table 6

Correlation of the three body segments of ses B^1 mosaics with their behaviour.

	CUTICLE GENOTYPE					
	head		thorax		abdomen	
	mutant	normal	mutant	normal	mutant	normal
BEHAVIOUR						
mutant	23	5	24	2	13	5
inconsistent	39	28	34	16	22	24
normal	6	33	6	20	3	16

Table 7

List of drugs which have a more pronounced effect on the viability of various stress-sensitive strains. The effects of the same drug on Oregon-R are also shown. Drugs were dissolved in water to make different concentrations before they were added to the instant Drosophila medium (boreal). Cultures with no drugs dissolved in the water served as controls.

		Ore-R		ses B[2]		ses B[1]		ses D[1]		ses E[1]	
		pupae/larvae %	adults/pupae %	pupae/larvae %	adults/pupae %	pupae/larvae %	adults/pupae %	pupae/larvae %	adults/pupae %	pupae/larvae %	adults/pupae %
Reserpine	.05	33.3	92.3	20.0	75.0	19.0	62.5				
	.1	54.1	80.0	4.8	100.0	2.5	100.0				
	.2	17.1	71.4	0.0	-	0.0	-				
	.4	2.9	100.0	0.0	-	0.0	-				
	.8	0.0	-	0.0	-	0.0	-				
Nicotine	.0156	83.7	97.2	81.0	73.0	88.0	97.0				
	.03125	94.3	97.0	64.0	80.0	97.0	94.0				
	.0625	71.4	88.6	0.0	-	74.0	100.0				
	.125	2.9	100.0	0.0	-	34.0	100.0				
	.25	0.0	-	0.0	-	6.0	100.0				
Pilo-	.2	72.2	66.7					75.0	70.0		
cárpine	.4	62.5	100.0					52.5	76.2		
nitrate	.8	60.0	100.0					35.0	85.7		
	1.6	0.0	-					29.3	91.7		
	3.2	0.0	-					44.7	29.4		
Carbamyl-	.75	73.8	96.8							82.1	91.3
choline	1.5	67.4	100.0							69.0	100.0
chloride	3.0	33.3	100.0							89.3	100.0
	6.0	0.0	-							60.7	70.6
	12.0	0.0	-							70.0	100.0
H_2O		87.2	91.2	94.9	94.6	75.0	100.0	85.0	94.1	57.1	91.7
control		72.1	90.3	78.4	93.1	80.0	96.9	94.9	94.6	96.4	92.6
no drugs		82.5	100.0	51.3	65.0			97.4	100.0	100.0	82.5
		50.0	84.0								

concentration of drug. Similarly, a mutant is less sensitive or more resistant to a particular drug if its larvae have a greater viability than Oregon-R at the same concentration of drug. Only mortality is examinied here. The behavioural effects of these drugs at concentrations lower than lethal dose were not studied.

The ses B^1 mutation is more sensitive to reserpine (reduce catecholamine transmitter storage) and is more resistant to nicotine (an agonist of acetylcholine).

The ses B^2 mutation is more sensitive to reserpine (same as the ses B^1 mutation) but unlike the ses B^1 mutation, it is more sensitive to nicotine as well.

ses D

Genetic Mosaic Studies: ses D^2 is not temperature-sensitive and is moderately sensitive to stress compared to the other mutants studied. Paralyzed ses D^2 flies normally recover within $1\frac{1}{2}$ minutes. When homozygous ses D^2 flies are under stress, all six legs are often paralyzed. However, it was noticed that when ses D^2 bilateral mosaics (one side of the fly having normal cuticle colour while the other side is totally mutant) are stressed, the mutant legs were often paralyzed while the wild type legs were never paralyzed. The other mosaics under stress, in which some of the 6 legs are of different phenotypes, often have their walking wild type legs dragging their paralyzed mutant ones. Thus the behaviour of each leg is independent of the others with behaviour and cuticular genotype having a high degree of concordance,

so the behaviour of each leg of the mosaic fly was scored independently. This allowed the focus mapping of the behaviour for each leg.

Analysis of 1608 legs of mosaics revealed that the behavioural focus of each leg has its own site within the presumptive nervous system of the blastoderm fate map. The 6 foci occupy 6 seperate, non-interacting sites in the blastoderm aligned with their respective legs (Fig. 6).

Drug Studies: ses D^1 is more resistant (less sensitive) to pilocarpine nitrate (an agonist of the cholinergic system). (Table 7) ses D^2 response did not differ from the Oregon-R wild type.

ses E

Characterization of TSPs: ses E^1 is the least stress-sensitive as paralyzed ses E^1 flies usually recover in a matter of 5 to 10 seconds. The temperature-sensitive lethal phenotype of ses E mutation enabled analysis of gene activity by temperature-shift experiments. Reciprocal single shift studies at 24-hour intervals from permissive to restrictive temperatures and vice versa revealed that ses E^1 has a TSP during the transition of 1st to 2nd instar larvae (Fig. 7). The lethal phase (LP) for this TSP is pupariation. At the restrictive temperature, few pupae form; most 3rd instar larvae wander around the vial or bottle and die on the sides without forming a pupal case. This TSP starts at approxiamtely 45 hours at 22°C after oviposition and ends at 117 hours, a duration of approximately 70 hours.

ses E^1 also has another TSP for lethality. To delineate

Figure 6. Fate map of ses D^2. a, antenna; anp, anterior notopleural bristle; hu, humerus; oc, ocellar; 1c, coxa 1; 2c, coxa 2; 3c, coxa 3; 3s, 3rd sternite; I, leg 1 focus; II, leg 2 focus; III, leg 3 focus.

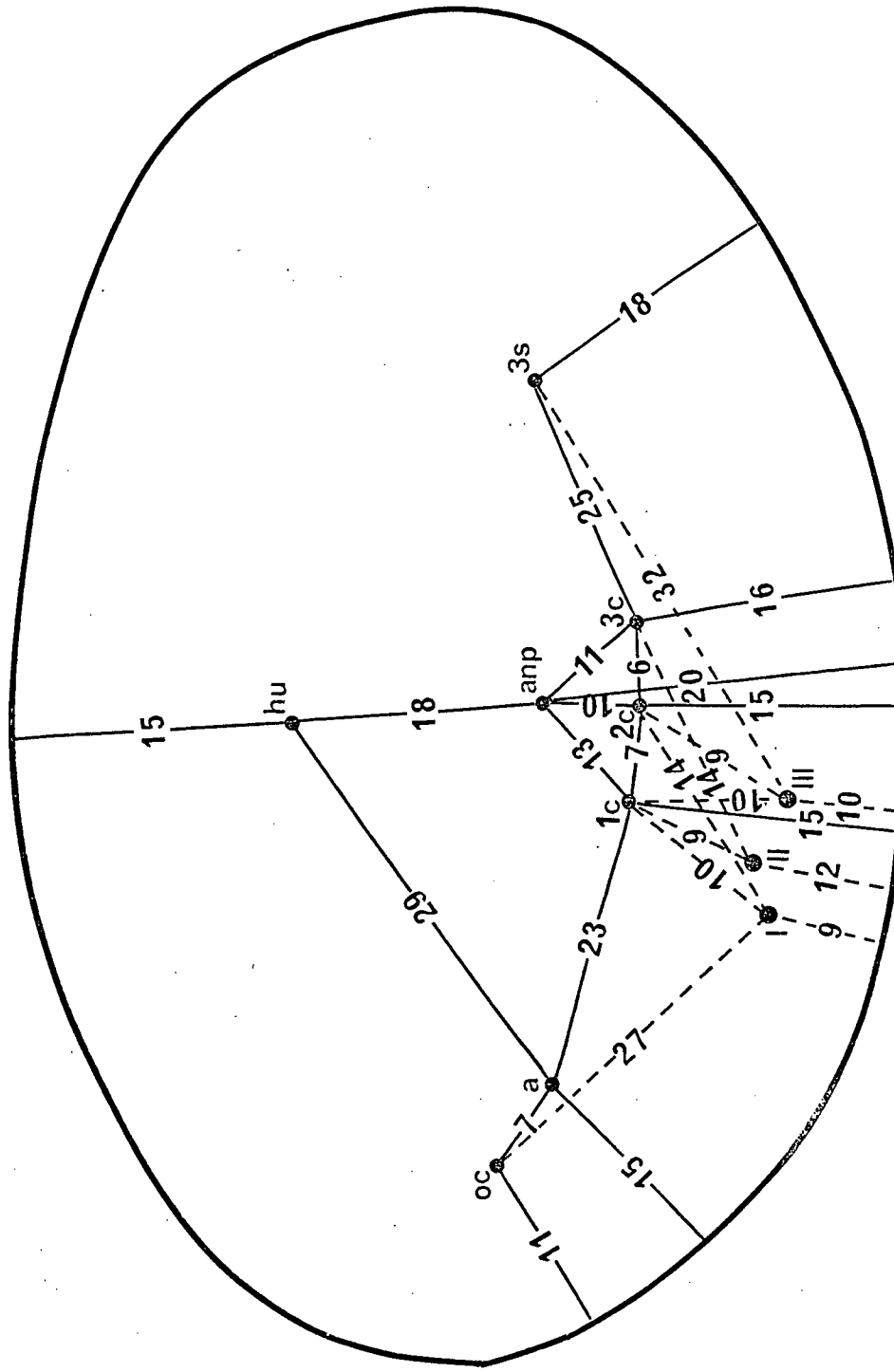
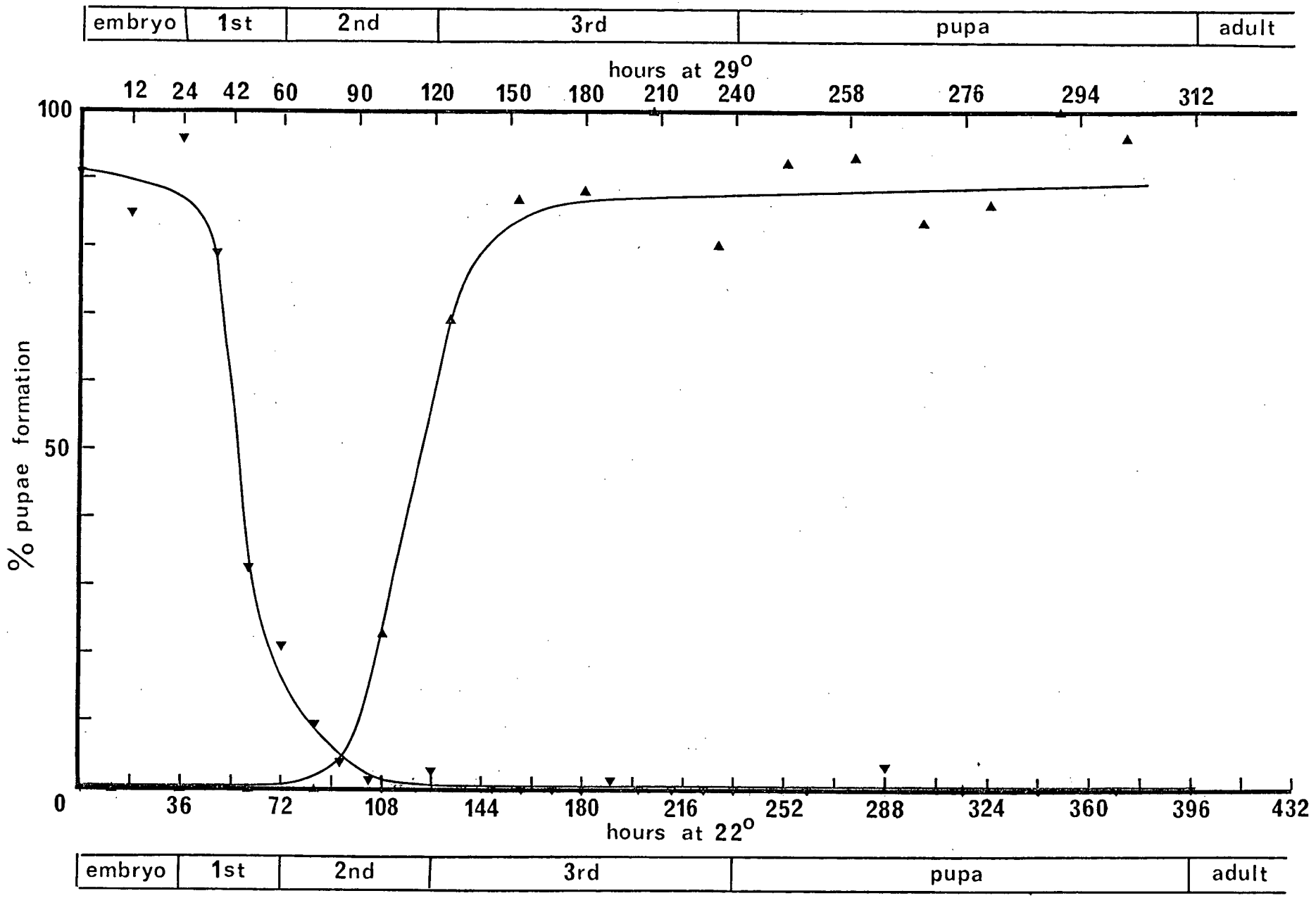

3s
18
25
32
3c
16
11
6
anp
10
20
15
hu
15
18
2c
13
7
14
9
1c
10
15
10
III
9
12
III
I
9
23
27
a
7
oc
15
11

Figure 7. Percentage of ses E^1 pupae formed after, ▲, shift-ups and ▼, shift-downs administered at different times during development. The developmental stages at 22°C and 29°C at the initiation of the shift are also shown at the bottom and at the top respectively.


embryo
1st
2nd
3rd
pupa
adult
hours at 29°
12 24 42 60 90 120 150 180 210 240 258 276 294 312
100
50
0
% pupae formation
36 72 108 144 180 216 252 288 324 360 396 432
hours at 22°
embryo
1st
2nd
3rd
pupa
adult

the start of this TSP, fourteen cultures were synchronized at egg collection, maintained at 22°C for 6 days in order to pass through the larval TSP, then shifted up to 29°C and maintained there. At 24-hour intervals, 2 cultures were shifted down to 22°C until all 14 cultures at 29°C had been transferred. This established heat pulses of varying duration. These heat pulses revealed that the second TSP starts at approximately 198 hours at 22°C. Standard shift-up experiments revealed this TSP ends at 288 hours at 22°C, a time corresponding to the early pupal stage (Fig. 8). Thus, ses E^1 has a 3rd instar larval-pupal TSP approximately 90 hours long.

To confirm the 3rd instar larval-pupal TSP and to examine the sensitivity of the 2 TSPs to heat-induced lethality, 24-hour heat pulses were administered at various times during development. No 24-hour heat pulse caused death of all developing flies. The most sensitive time interval during the second TSP was at the 3rd instar-pupal transition stage during which 24-hour heat pulses significantly reduced the number of adults from 85% in controls to 15% (Fig. 9). There were no phenotypic abnormalities associated with these 15% survivors. The 24-hour pulse experiments did not reveal the larval TSP shown by the shift experiments, thereby indicating that a longer portion of this TSP must be spent at restrictive temperatures to induce death.

Genetic Mosaic Studies: Apart from the stress-sensitive phenotype, ses E is also a ts drop-dead. The 230 mosaics recovered

Figure 8. Percentage of ses E^1 adults eclosed after, Δ, shift-ups and ∇, shift-downs administered at different times during development. The developmental stages at 22°C and 29°C at the initiation of the shift are also shown at the bottom and at the top respectively.

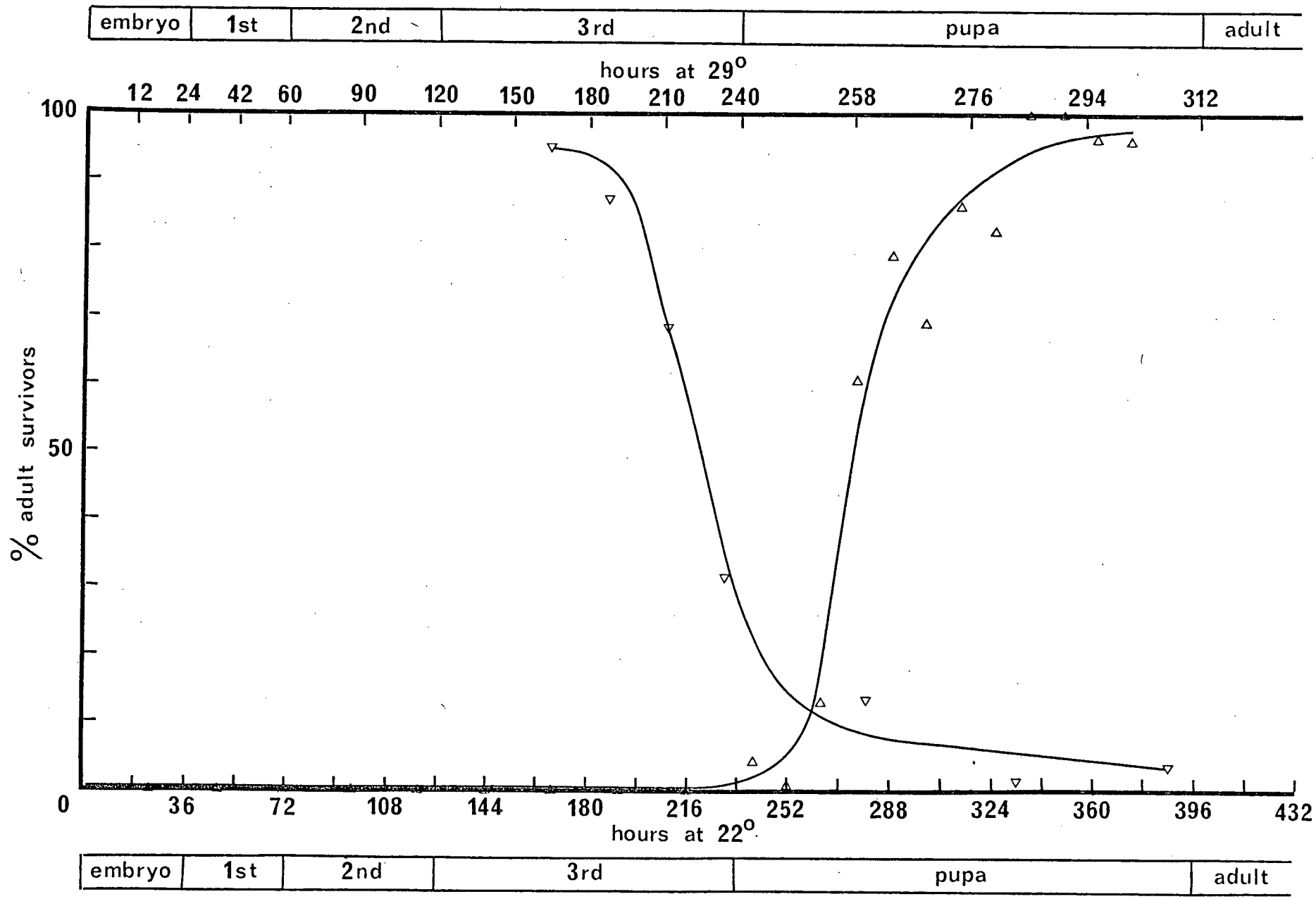
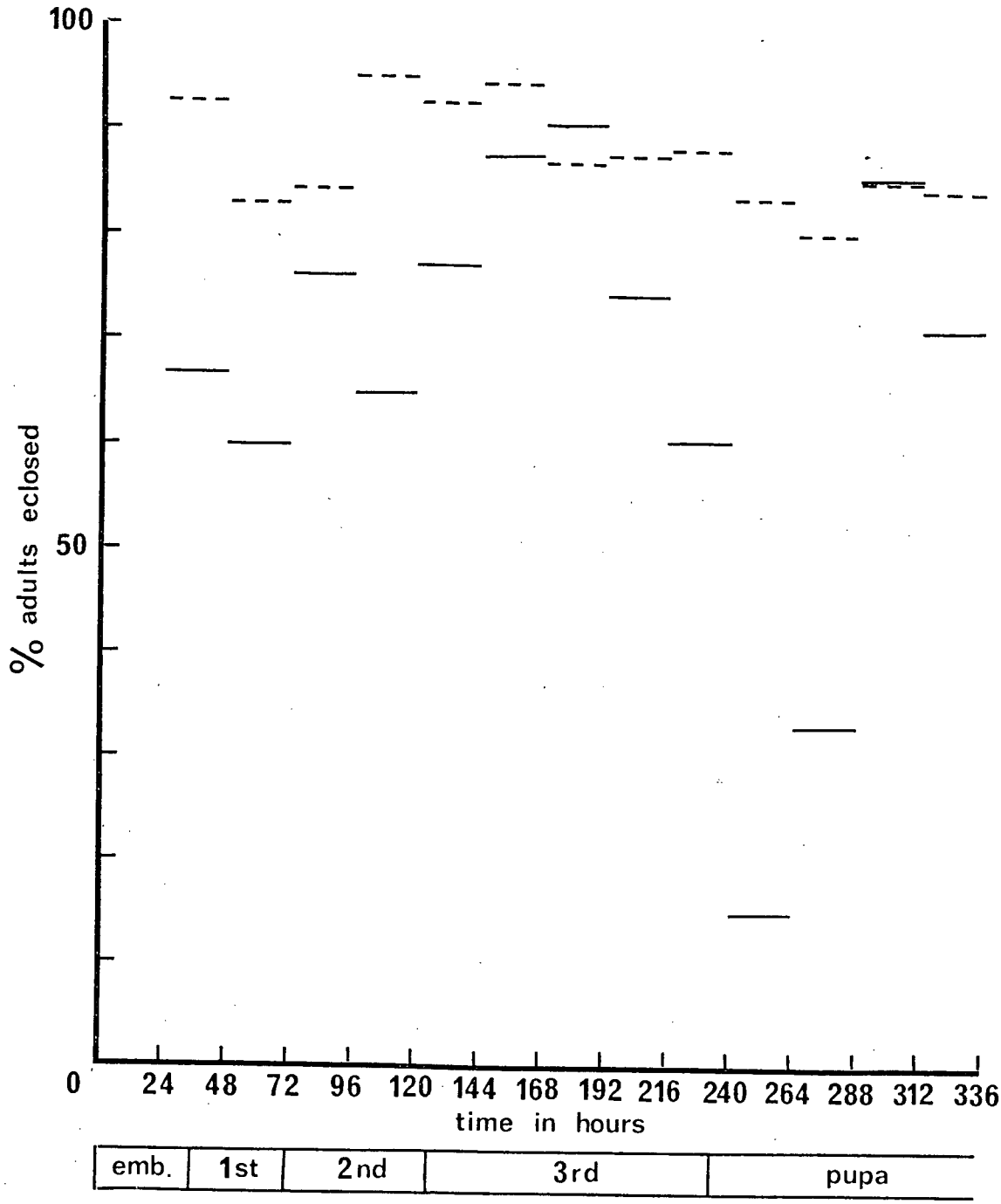

embryo
1st
2nd
3rd
pupa
adult
hours at 29°
12 24 42 60 90 120 150 180 210 240 258 276 294 312
100
50
0
% adult survivors
36 72 108 144 180 216 252 288 324 360 396 432
hours at 22°
embryo
1st
2nd
3rd
pupa
adult

Figure 9. Percentage of, ——, ses E^1 adults and – –, Oregon-R adults eclosed after 12 hour heat pulses (29°C) administered at different times during development. The developmental stages at the time of the pulse are shown at the bottom. The lengths of the lines represent the duration of the pulses at 29°C.


100
50
0
% adults eclosed
24 48 72 96 120 144 168 192 216 240 264 288 312 336
time in hours
emb.
1st
2nd
3rd
pupa

were observed for behaviour and viability at 29°C. During the course of observation, it was noted that individual legs of mosaic flies became paralyzed. Hence, the mosaic data of ses E^1 can be examined in two ways. Assuming the different phenotypes of ses E^1 are a consequence of a same point mutation, then both adult lethality and stress-sensitivity may be due to effects on the same tissues or on two different tissues. By focus mapping both drop-dead and stress-sensitive phenotypes, these alternatives might be distinguished. Unfortunately, exposing the mosaics to high temperature to test the paralytic behaviour caused death in many flies before paralysis could be observed. Thus, the occurence of lethal phenotype before leg paralysis obscurred quantitative assessment of leg phenotype. As a result of this, only 27% of the legs were judged to have become paralyzed before the time of death and conventional focus mapping using the leg phenotype was not possible.

Examination of the survival curve (Fig. 22, Chapter 3) shows that 73% of the mosaics died during the first 13 days of incubation at 29°C. Specifically, most of these died between days 5 and 13, after which time the survival curve levels off. Conventional mosaic analysis for the lethal phenotype was undertaken, assuming that this 73% fraction represents mosaics with a lethal focus (or foci). Inspection of the data showed that 24 of 25 mosaics, which were bilaterally mosaic for two of the three body segments, died prematurely. On the basis of these data, the ses E^1 mutant foci are judged to be domineering. Using the mathematical analyses derived for a domineering model (Hotta and Benzer,

1972), the foci were localized relative to cuticle markers. The position shown in Figure 10 was determined to best fit the data. This position places the foci near the posterior regions of the presumptive nervous system. It can be seen, however, that discrepancies for several of the distances appear in this fate map. Thus, it seems inconsistent that the distance between the humeral and ses E foci (17 sturts) should be less than the distance between the humeral and anterior notopleural bristle (24 sturts). A possible explanation for this inconsistancy is that the focus is diffuse, that is, it occupies a relatively large area of the blastoderm. It is also complex in that there are 2 foci, one on each side of the blastoderm. The foci are bilaterally domineering which means that only one of these two foci need be mutant for the mutant behavioural phenotype to be fully expressed. This is in contrast to bilaterally submissive foci in which both foci must be mutant in order to confer a mutant behavioural phenotype. Using the same calculation which had placed the foci in the vicinity of coxa 2 and coxa 3, the distance between the left and right foci is found to be about 50 sturts. This would place them in the middle of the blastoderm fate map. However, if the focus is diffuse, the chance that it will have a mixed genotype (that is, $\frac{1}{2}$ mutant, $\frac{1}{2}$ normal) will increase. And if a small amount of mutant tissue is sufficient to produce a mutant phenotype, the chances of the focus appearing mutant will increase. Since ses E^1 foci are bilateral and domineering, the portion of mosaics exhibiting mutant behaviour should be large. As Hotta and Benzer (1972) show, this

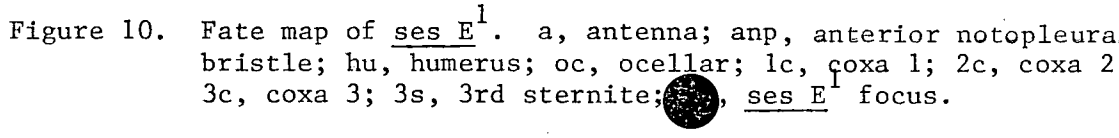
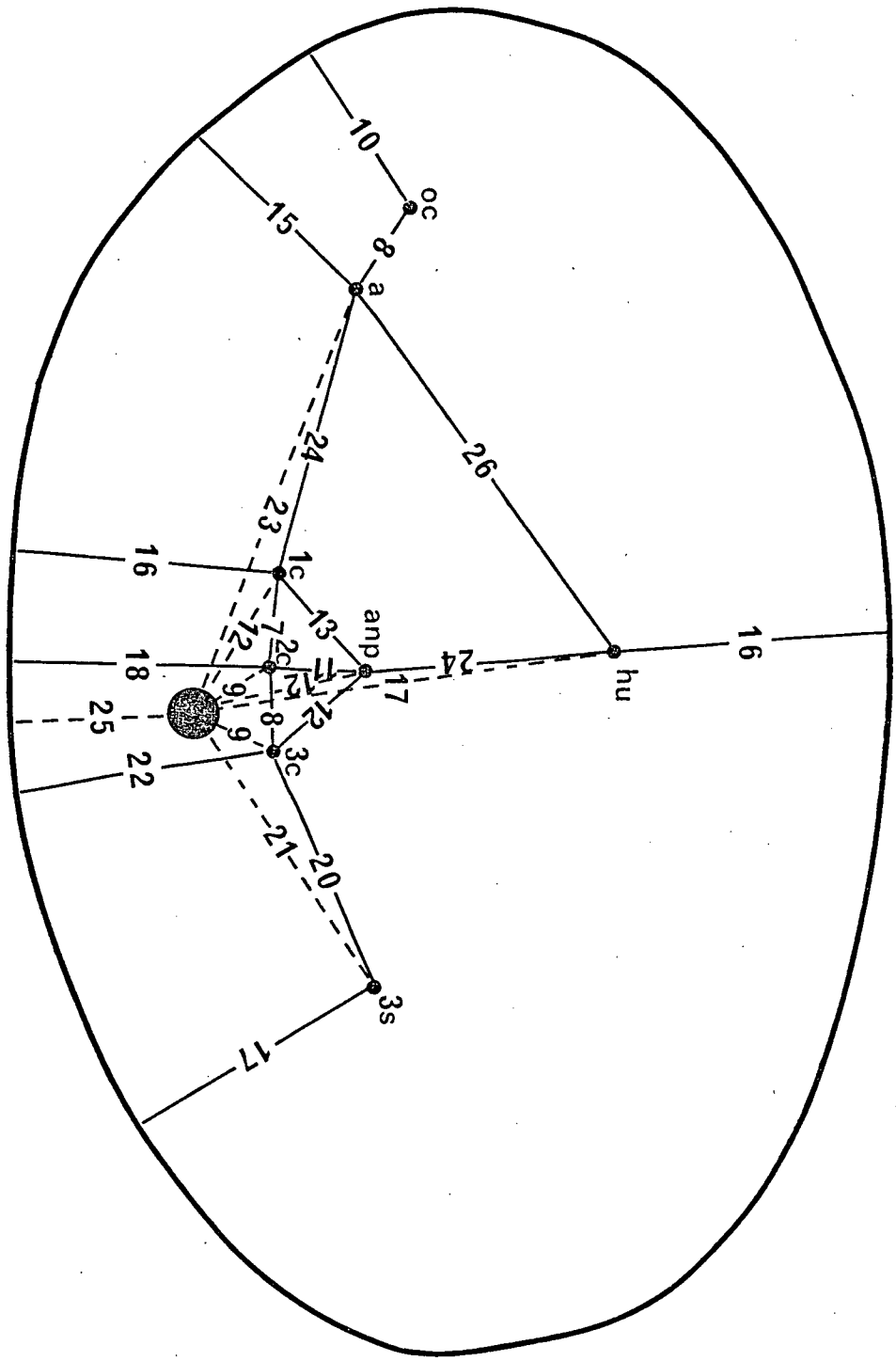


Figure 10. Fate map of ses E^1. a, antenna; anp, anterior notopleural bristle; hu, humerus; oc, ocellar; 1c, coxa 1; 2c, coxa 2; 3c, coxa 3; 3s, 3rd sternite; ●, ses E^1 focus.


oc
a
anp
hu
1c
2c
3c
3s
10
15
8
24
23
26
16
13
12
7
11
17
18
25
22
9
21
20
17

would ultimately increase the distance between 2 bilaterally domineering foci.

As noted before, conventional focus mapping of the leg paralysis phenotype of ses E^1 could bot be performed due to the early onset of lethal phenotype. However, using the 27% of the legs that showed the paralytic phenotype consistently before death, it was possible to correlate the phenotype of the leg cuticle with their paralysis. This showed that leg paralysis did not always occur in a mutant leg and implies that the foci for leg paralysis and the thoracic cuticle are separable. The correlation analysis did show, however, that a paralyzed prothoracic leg was most commonly associated with mutant tissue in the anterior notopleural bristle (ANP), coxae of the first, second and third legs in that order (Table 8). Likewise, paralysis of legs 2 and 3 were more often associated with mutant cuticle in the coxae of each respective leg, than with mutant cuticle in the respective tarsi or other thoracic parts.

These data suggest that the foci for leg paralysis lie somewhere below the thoracic hypoderm on the blastula fate map, in the general area where the adult lethal foci were localized.

Therefore, using both modified and conventional mosaic analyses, it is possible to map out the same area in the ventral nervous system of the fly which is believed to be the primary focus for the stress-sensitive and drop-dead phenotypes.

Drug Studies: Results (Table 7) indicated that ses E^1 is

TABLE 8

The distance of ses E[1] focus with respect to various surface landmarks. The distances are ranked from 1 to 10 with 1 being closest to and 10 being furthest away from the focus.

Distance from focus to	leg 1 ranking	leg 2 ranking	leg 3 ranking	Total	combined ranking
Antenna	8	10	9	27	9
Humerus	9	9	10	28	10
ANP Bristle	1	4	4	9	3
Coxa 1	2.5	7	7	16.5	5
Coxa 2	2.5	2	3	7.5	2
Coxa 3	4	1	1	6	1
Tarsus 1	6.5	6	6	18.5	7
Tarsus 2	6.5	5	5	16.5	5
Tarsus 3	5	3	2	10	4
Sternite 3	10	8	8	26	8

more resistant to an agonist of cholinergic system, carbamylcholine chloride.

IV. Discussion

Single gene mutations which affect the motor activity of flies have proven to be useful in pinpointing the genetic regulation of behaviour and its neuromuscular origin. While physiologists study the direct effect of stress on normal insects, the present study has made use of mutations which exaggerate sensitivity to stress to the point of paralysis to allow us to pinpoint the cells affected for further physiological studies.

Temperature-sensitivity is a useful property of the ses B mutation. Developmental studies indicate that the ses B mutant gene product is required during the larval stage and possibly in the ovary during oogenesis. This conclusion is reached after temperature-shift studies revealed that a ts maternal-effect exists for ses B^2. Eggs maintained at 29°C from female parents which have been kept at 29°C for more than 3 days do not develop into adults (less than 10%). Since approximately 3 days are required for complete oogenesis from stage 1 to stage 14 oocyte (see King, 1970 for definitions of the stages) in Drosophila, the results could suggest that a thermolabile product which is essential throughout the later stages of development is synthesized by and incorporated into the early oocyte. However, if these eggs were shifted down immediately after the egg is laid, about 50% of them develop into adults. This indicates that either the thermolabile product can be reactivated by a change of temperature or the

embryonic cells make enough product at the permissive temperature to "rescue" the embryos. The latter possibility is supported by survival of heterozygous females from homozygous mothers at 29°C. It has also been shown that when ses B embryos or 1st instar larvae are exposed to 29°C heat pulses, larval development is prolonged and in some instances, development is arrested in the 3rd instar. In contrast, exposure of 2nd or 3rd instar larvae to such temperatures results in little prolonged 3rd instar larval period. Temperature-shift studies also indicated a 3rd instar-pupal TSP and that shift downs as late as 2nd instar are capable of forming adults. These results indicate that 2nd instar larvae which had been raised at 29°C are rescuable by allowing them to develop throughout the 3rd instar-pupal TSP at 22°C. These observations, together with the result from maternal-effect studies (which indicate that +/ses B^2 heterozygotes derived from ses B^2/ses B^2 mothers are capable of surviving) suggest that ses B^2 gene product is synthesized and required during oogenesis as well as embryonic, 1st instar and 3rd instar-pupal stage. These TSPs are related in such a way that the reduced larval viability due to exposure to 29°C at earlier TSPs can be alleviated by further developing them at 22°C during later TSPs. Many examples of sex-linked maternal-effect mutations exist in Drosophila including sonless (Colaianne and Bell, 1972), deep orange (Garen and Gehring, 1972), cinnamon (Baker, 1973), fused and rudimentary (Fausto-Sterling, 1971), murky and singed (Mohler, 1977), and 1(1)E25^{ts} (Tarasoff and Suzuki, 1970). ses B^2 is peculiar in that its ts gene

product is not only important prior to and at the embryonic stage, but during later stages as well. Also, it differs from other non-conditional mutants in that its gene product is partially rescuable by avoiding subsequent development at the restrictive temperature.

Although mosaic analysis had suggested that mutant tissues in the head and the thorax will most likely confer a mutant behavioural phenotype, the vast amount of tissues in these regions make it almost impossible to pinpoint the abnormal behaviour to a specific tissue. On the other hand, the presence of a large number of mosaics with inconsistent behaviour (Table 6) could suggest that the inconsistency is actually an illusion of intermediate behaviour between the two extreme phenotypes. Thus they were classified as mutant on one day and normal on another. This, together with the fact that there is only a minority of mosaics with completely mutant thoraxes showing mutant behaviour (for example, of the 64 mutant thoraxes (Table 6), 24 had mutant behaviour, 34 had inconsistent behaviour and 6 had normal behaviour.) suggest that ses B gene could act non-autonomously, that is, the mutant gene does not act directly on the motor regions of the thoracic ganglia, but indirectly through a circulating substance produced elsewhere in the fly. In fact, in the American cockroach, Periplaneta americana, physiological studies have shown that such a circulating substance called Factor S does exist in the insect's haemolymph and is released in larger amounts during stress (Beament, 1958; Helsop and Ray, 1959; Sternburg, 1963; Davey, 1964; Samaranayaka, 1974). At low concentrations,

Factor S initiates a state of hyperactivity in the nervous system of the organism while at higher concentrations (which is the case when the organism is exposed to prolonged periods of stress or higher level of stress), Factor S causes a neural blockade (Cook, 1967; Cook, de la Cuesta and Pomonis, 1969). Therefore, it is likely that a circulating substance analogous to that in the cockroach exists in Drosophila, bearing in mind that there are still possibilities of mutant receptor cells being extra sensitive to Factor S and/or mutant cells having a lower threshold for release of Factor S.

These results point to future pursuits to test the gene autonomy of ses B. An easy way would be to perform reciprocal ovary transplants between wild type host and mutant ovaries, and vice versa. If the ses B gene does act non-autonomously during oogenesis, then wild type ovaries in ses B host will be predicted to be temperature-sensitive, and ses B ovaries in wild type host will be predicted to produce eggs at 29°C that will give rise to adults.

The see B mutation is also more sensitive to reserpine. The pharmacological action of reserpine is to release (and thus reduce) catecholamine (a biogenic amine) storage in insects (Bertler, Carlsson and Rosengren, 1956; Burn and Rand, 1957, 1958, 1959a,b. Burn, 1960). In spite of several efforts by Cook (1967) and Sternburg and his co-workers (Sternburg, Chang and Kearns, 1959), the exact biochemical determination of Factor S was unsuccessful. However, both studies concluded that Factor S is a biogenic amine. Biogenic amines in

insects are apparently stored and released to act in at least two ways: (1) from conventional neurons where their effects are localized to the immediate synaptic region, (2) released from neurosecretory organs where their effects may be more generalized. The extra sensitivity of ses B to reserpine therefore suggests that stress paralysis in Drosophila could be mediated by a biogenic amine. Based on this result and the fact that the paralysis-causing agent is a circulating biogenic amine, the non-autonomous nature of the ses B gene becomes more probable.

Thus, in addition to its stress-sensitivity effects, the ses B mutation also affects oogenesis, embryogenesis and pupariation. It represses pupariation by prolonging the 3rd instar larval period for as long as 3 weeks before death commences. The present studies suggest that ses B could be an excellent candidate for studying hormones in insects because hormones are known to play important roles in fertility, development and metabolism. Before any further speculation, more experiments are needed. For example, if the ses B gene express itself non-autonomously, then perhaps quantitative analysis on the stress factor by extraction and subsequent assay for its activity by injection into normal flies, or application to isolated insect nerve preparation (Cook, de la Cuesta and Pomonis, 1969), or measurement of its effect on sensitizing larvae to a pupation hormone, ecdysone.

One of the other two stress-sensitive mutations studied is ses D^2. Mosaic analysis on ses D^2 suggested a neural dysfunction. Mosaic for ses D^2 exhibit independent behaviour of each leg upon

stress. This differs from ses B^1 mosaics where the effect is all or none, that is, either all 6 legs are paralyzed or they are not. The faci for ses D^2 map to the same area of the fate map as those for Hk^1 (Hotta and Benzer, 1972). Hk^1 mutants over-react to stimuli such as sudden movements and often shake their legs vigorously under ether anaethesia. The embryonic foci for Hk^1 lie near their corresponding legs and each are located at positions ventral to their adult cuticular sites. This area closely corresponds to the region of the blastoderm destined to form the ventral nervous system. This interpretation was further supported by electrophysiological studies (Ikeda and Kaplan, 1974) which showed that all action potentials of a certain type of neuron in the thoracic ganglion are preceded by slowly rising depolarizations, hence the rhythmic bursts of activity is observed. The frequency of this endogenous activity is also found to be similar to that of the shaking legs. Hence, the congruence of the ses D foci with those of Hk^1 suggests that ses D paralysis could be due to hyperactivity in the thoracic ganglia as well.

The behavioural phenotype of the ses D^2 mutant resembles that of another bang-sensitive mutant, bas, whose embryonic focus is in the nervous system (Feitelson and Hall, 1975). Further electrophysiological studies on this mutant revealed that there is an abnormal facilitation pattern after mild stimulation. Facilitation is an enhanced post-synaptic response caused by one or more previous synaptic transmissions. The neuronal facilitation observed in bas develops much

faster and decays much slower and is associated with a prolonged release of transmitter at the pre-synaptic neuron caused by unknown reasons (Jan and Jan, 1978). The insect's hyperactivity as a preliminary response to stress is known to be a phenomenon of facilitation (Helsop and Ray, 1959; Sternburg, Chang and Kearns, 1959; Cook, 1967) where sustain levels of it can produce paralysis (Cook, de la Cuesta and Pomonis, 1969).

Therefore, the similar location of foci of ses D^2, Hk^1 and bas and the resemblance in phenotypes of bas and ses D^2 suggest that there could be an enhanced or a faster development of facilitation and/or a prolonged time course of facilitation in ses D^2 as well. The relationship of the pilocarpine nitrate (an agonist of acetylcholine) resistance to the abnormal response to stress of ses D, if any, is unknown. However, if the ses D defect is due to abnormal facilitation caused by prolonged release of transmitter at the neuromuscular junction, then these results could suggest the existence of an inhibitory interneuron acting on the motor neuron via the cholinergic system. This conclusion is reached after several independent efforts fail to provide evidences of cholinergic neuromuscular junctions in insects (Hildebrand, Hall and Osmond, 1979; Eldefrawi, 1976; Jan and Jan, 1976).

The last of the stress-sensitive mutations studied, ses E^1, had pleiotropic effects including stress-sensitive and drop-dead phenotypes. Although conventional fate mapping with mosaics did not firmly position an embryonic focus for the adult lethal phenotype, it did

suggest a general region of the blastoderm which is more neural than mesodermal in origin. The focus for ses E^1 is quite diffuse and is located in an area of the blastoderm destined to form the thoracic ganglia. The importance of the integrity of the thoracic ganglia in providing a proper response to stress has already been emphasized. Therefore, in addition to its effect on stress-induced paralysis, the thoracic ganglia might be involved in the drop-dead phenotype as well. It should be noted that the temperature-sensitive lethality of ses E could be explained by not only being extremely sensitive to mechanical stress but to other forms of stress such as heat as well.

Drug studies indicated that ses E^1 is more resistant to another agonist of acetylcholine, carbamylcholine chloride (carbachol). Therefore, like ses D, a cholinergic system suppressing the stress-sensitive phenotype and, in this case, the drop-dead phenotype as well cannot be ruled out. A better understanding on the neural defect of these mutants is to isolate the cholinergic receptors (Eldefrawi, 1976) and perform a study on the binding properties of different neurotransmitters to their receptors. If such studies indicate the binding properties of cholinergic receptors are different, then defects other than abnormalities at the neuromuscular jucntions must operate in these stress-sensitive mutations.

In summary, there are at least two types of stress-sensitive mutations: one is generalized, affecting a large amount of neural tissue while the other is more localized, affecting a single neuron controlling leg movements. Hence, one has to be very selective in choosing the

mutant for further studies because stress-sensitive mutations are believed to act via three ways (Feitelson and Hall, 1975; Homyk, 1977): (1) mutant cells are hypersensitive to the stress factor, (2) mutant cells release an excessive amount of stress factor during stress, and (3) mutant cells fail to produce an enzyme or enough enzyme which normally inactivate the factor. The present study has localized the defects of several stress-sensitive mutations to their blastoderm fate map and has delineated TSPs for mutations with ts developmental phenotypes. More techniques in anatomy, biochemistry and histochemistry are therefore necessary to determine the specific role of genes in development and provide interesting relationships between genes, development and behaviour.

CHAPTER 3

DROP-DEAD MUTATIONS

I. Introduction

The components of behaviour, from the sensory receptors in the peripheral nervous system to the central nervous system and back out via the effector organs are assembled under the direction of genes. Therefore, any alteration in these components by mutations will result in death or abnormal behaviour. Hence, by selection of mutants on the basis of their abnormal behaviour, specific components of the nervous system can be analyzed. One group of mutations with a stress-sensitive (ses) phenotype has already been described. The second class studied for this thesis is characterized by death of adults (drop-dead) at restrictive temperatures.

Other temperature-sensitive adult drop-dead mutations have been reported (Tarasoff and Suzuki, 1968). The first adult drop-dead mutant recovered in the present study was isolated as a recessive ts lethal on the X chromosome and only later, the ts adult drop-dead (*add* A^{ts1}) phenotype was found. A second ts drop-dead mutant, *ses E*, was found among flies recovered initially as stress-sensitive. Non-conditional mutations with an adult lethal phenotype, including *drd* (Hotta and Benzer, 1972), *dmd* (Flanagan, 1977) and *l(4)18* (Hochman, 1973) have been described. They all develop and eclose normally but die precociously soon after eclosion. Analysis of genetic mosaics

showed that the primary lethal focus of drd lies in the brain tissue and of dmd is in the thoracic ganglion. Hotta and Benzer (1972) were able to confirm their conclusions based on mosaic analysis and by histological work which showed that all drd flies have degenerated brain tissue. Thus, the drop-dead phenotype may prove to be a useful way to identify neural defects.

The temperature-sensitive lethal phenotypes of add A^{ts1} and ses E^{1} provide advantages over similar mutations with non-conditional lethal phenotypes (Suzuki, 1970). First, the ts nature of the lethal phenotype allows one to perform shift and pulse experiments between the permissive and non-permissive (restrictive) temperatures to determine the temperature-sensitive periods (TSPs). A TSP is a period during which development at the non-permissive temperature results in the mutant phenotype, in this case lethality. TSPs are generally regarded as the time of synthesis and/or function of a mutant gene product (Suzuki, 1970).

In the present case, temperature-shift studies revealed that the add A mutation has TSPs in the embryonic and late larval to early pupal stages as well as in the adult stage. Similarly, the ses E mutation was found to have TSPs in the mid-first to mid-second larval instars, mid-third larval instar to early pupal stage and of course, the adult stage. Furthermore, quantitative recovery and examination of adult mosaics for lethality and behaviour at the non-permissive temperature, allowed the localization of the add A adult

lethal phenotype to foci in the mesoderm and suggested that the foci for the ses E phenotype are most likely in the ventral nervous system.

II. Materials and Methods

A complete description of the isolation and properties of *add A*^{ts1} and *ses E*^{1} mutations used in this study has been reported (Homyk and Sheppard, 1977; Homyk, Szidonya and Suzuki, 1980). The map positions of the genes are: *add A*, 1-20.9; *ses E*, 1-27.7. Except for the adult ts lethal characteristic, *add A*^{ts1} mutants have developmental rates, behavioural activity and longevity identical to Oregon-R at 22°C. In contrast, *ses E*^{1} flies are detectably less active and develop much more slowly than Oregon-R. As well, flies carrying either *add A*^{ts1} or *ses E*^{1} have ts developmental lethal phenotypes.

Unless otherwise stated, all culturing conditions, heat pulses, temperature shifts, drug studies and genetic mosaic analyses are identical to those described in Chapter 2.

Examination of adult drop-dead behaviour

add A^{ts1}, *ses E*^{1} and Oregon-R wild type flies of different ages were shifted to 29°C and each day, their viability at 29°C was examined by recording the number of survivors left in each vial. Each vial contained 10 flies of one age group. Approximately 300 flies divided equally into three age groups were used with the exception of Oregon-R where only 100 flies of one age group was used.

Examination of embryonic development

In order to determine the nature of embryonic lethality of *add A*^{ts1}, embryos were photographed at 2-hour intervals beginning from

oviposition and continued until hatching. Eggs were collected within one-hour periods and dechorionated in 3% NaOCl (50% bleach). The embryos were then laid on the slides which were fixed on dry ice and photographed using bright field illumination. Stains were not used. However, a polarizing light filter was used to aid in observation of the muscles which will be biregringent under such conditions. All pictures were made with Kodak Panatomic-X film in a Carl Zeiss photomicroscope with a built-in camera set at the automatic exposure setting. The exposed film was developed and printed according to the manufacturer's specifications.

III. Results

add A^{ts1}

Characterization of adult drop-dead behaviour: When add A^{ts1} flies were shifted to 29°C, almost 95% died within 24 hours (Fig. 11) whereas only 7% mortality rate was observed for Oregon-R flies after 20 days at 29 C (Fig. 12).

Characterization of TSPs: TSPs of this mutant were initially determined by studying the consequences of 6-hour heat (29°C) pulses administered at different developmental stages. Anytime during the first 24 hours (embryo stage), such pulses significantly reduce egg hatchability (Fig. 13). More than 75% of add A^{ts1} eggs kept at 22°C hatch (data from shift studies, Fig. 15) whereas as few as 9% of heat pulsed embryos survive. In contrast, hatchability of Oregon-R eggs is unaffected by temperature; more than 75% of the eggs usually hatch.

The effect of heat pulses on post-embryonic development was monitored by calculating the fraction of hatched eggs which develop into adults. The data (fig, 14) show that a 6-hour heat pulse administered near the end of embryonic development or during the early 1st instar larval stage reduces the frequency of adult survivors. The same data also hint that additional TSPs exist at the mid-third instar stage (120 hours at 22°C) and early pupal stage (216 hours at 22°C).

Shift studies were performed to identify and describe the

Figure 11. Survival of △, 1 day old; ○, 5 day old; □, 10 day old *add A*^{tsl} adults at 29°C and , 1-5 day old *add A*^{tsl} adults at 22°C.

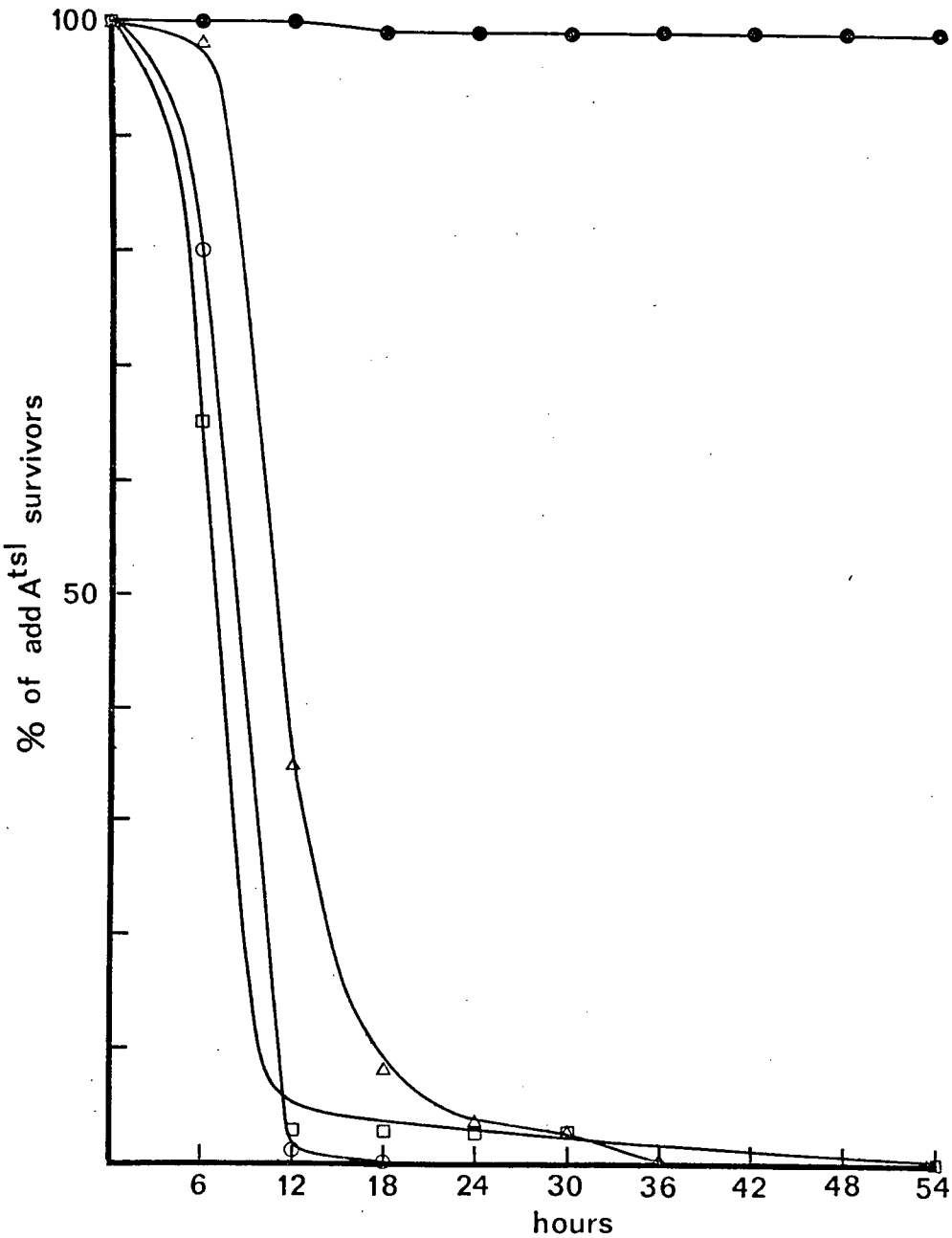

100
50
% of add A^tsl survivors
6
12
18
24
30
36
42
48
54
hours

Figure 12. Survival of 1-5 day old Oregon-R adults at 29°C.

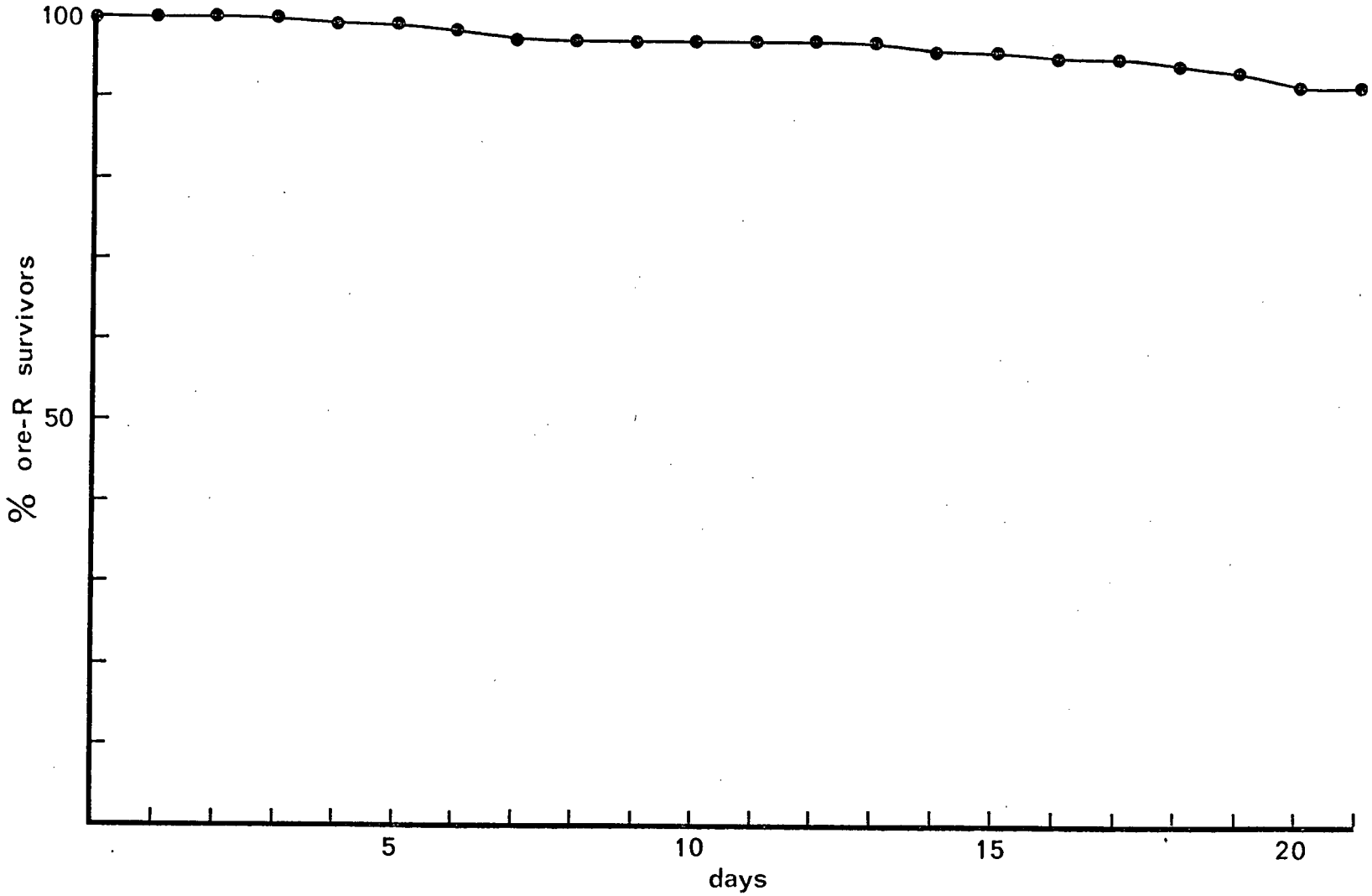

100
50
% ore-R survivors
5
10
15
20
days

Figure 13. Percentage of, ——, add A^{ts1} eggs; and, - - -, Oregon-R eggs that hatch after 6-hour heat pulses (29°C) administered at different times during development. The lengths of the bar indicate the duration of the pulses at 29°C. Approximately 100 eggs were used for each strain. Both strains have identical times for embryonic development.

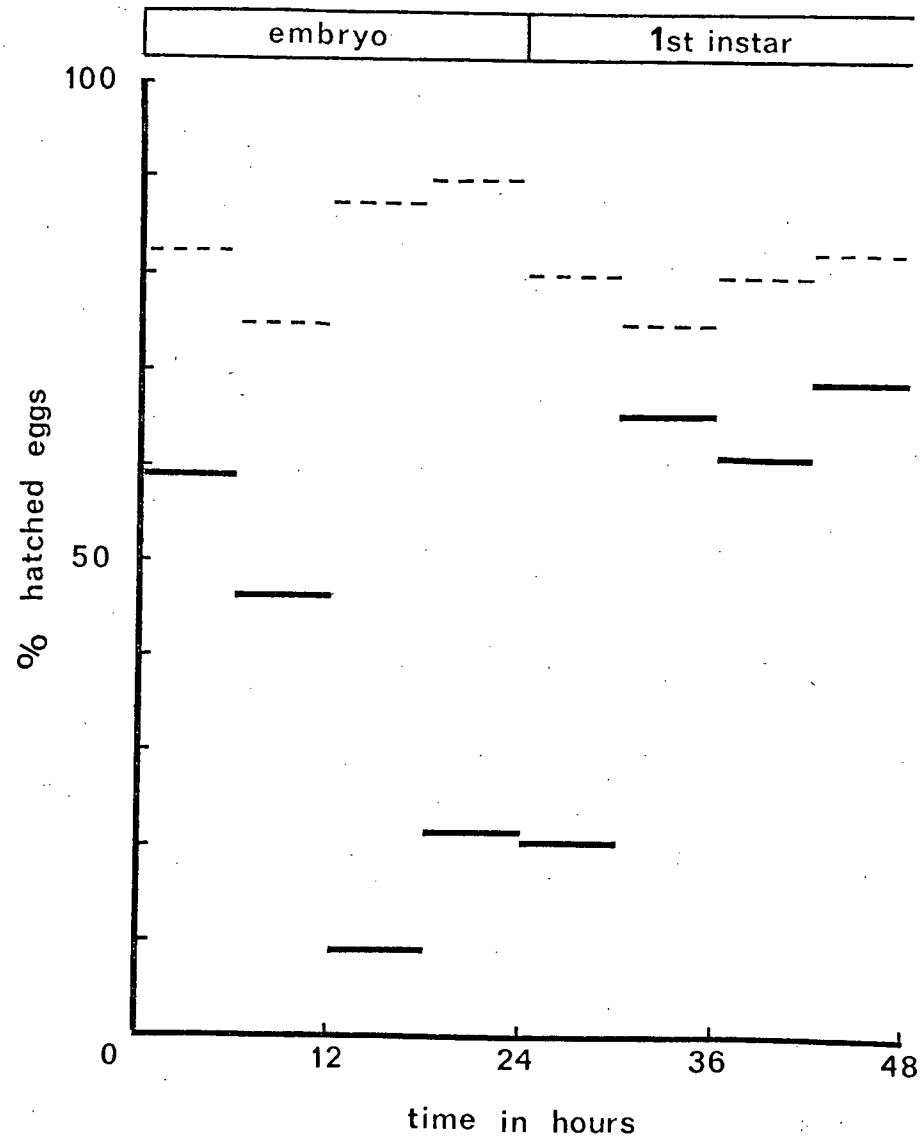

embryo
1st instar
100
50
0
% hatched eggs
12
24
36
48
time in hours

Figure 14. Percentage of <u>add A</u>^{ts1} eggs which yield adults after 6 hour heat pulses (29°C) administered at different times during development. The developmental stages at 22°C are shown at the bottom. Approximately 100 eggs were used.

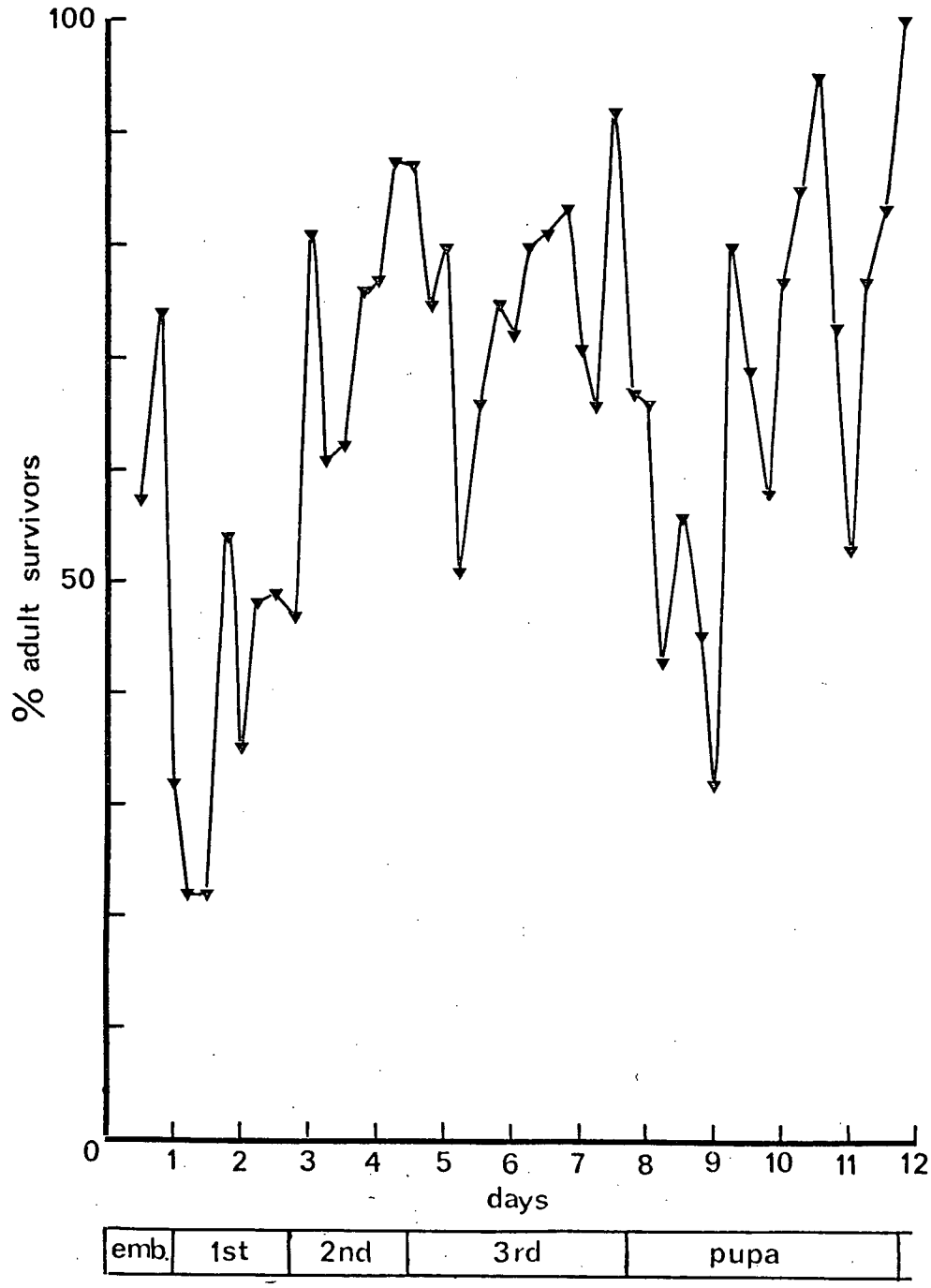
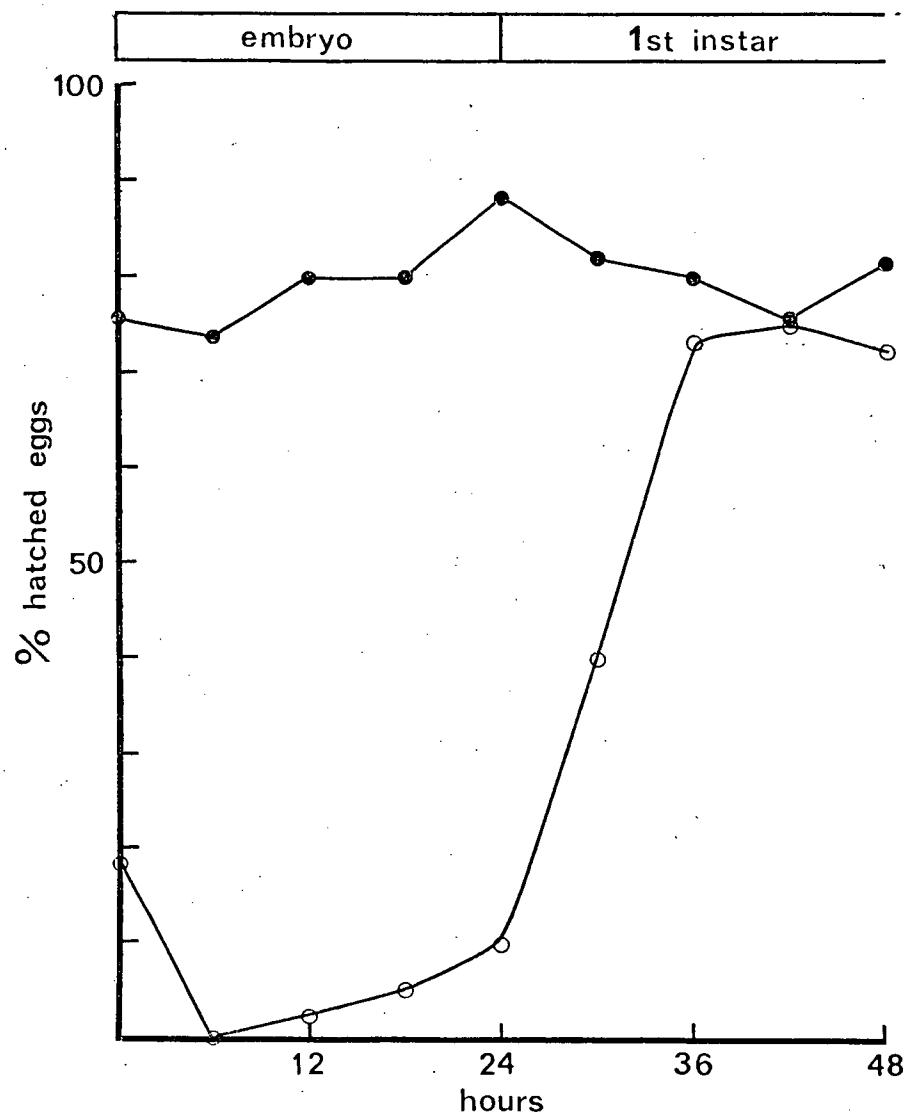

100
50
0
% adult survivors
1
2
3
4
5
6
7
8
9
10
11
12
days
emb.
1st
2nd
3rd
pupa

Figure 15. Percentage of, o—o, add A^{ts1} eggs which hatch after successive shift-ups administered at different times during development and, ●—●, control add A^{ts1} eggs which hatch after developing at 22°C throughout. Approximately 100 eggs were used for each experiment. Developmental rates did not differ in both experiments.

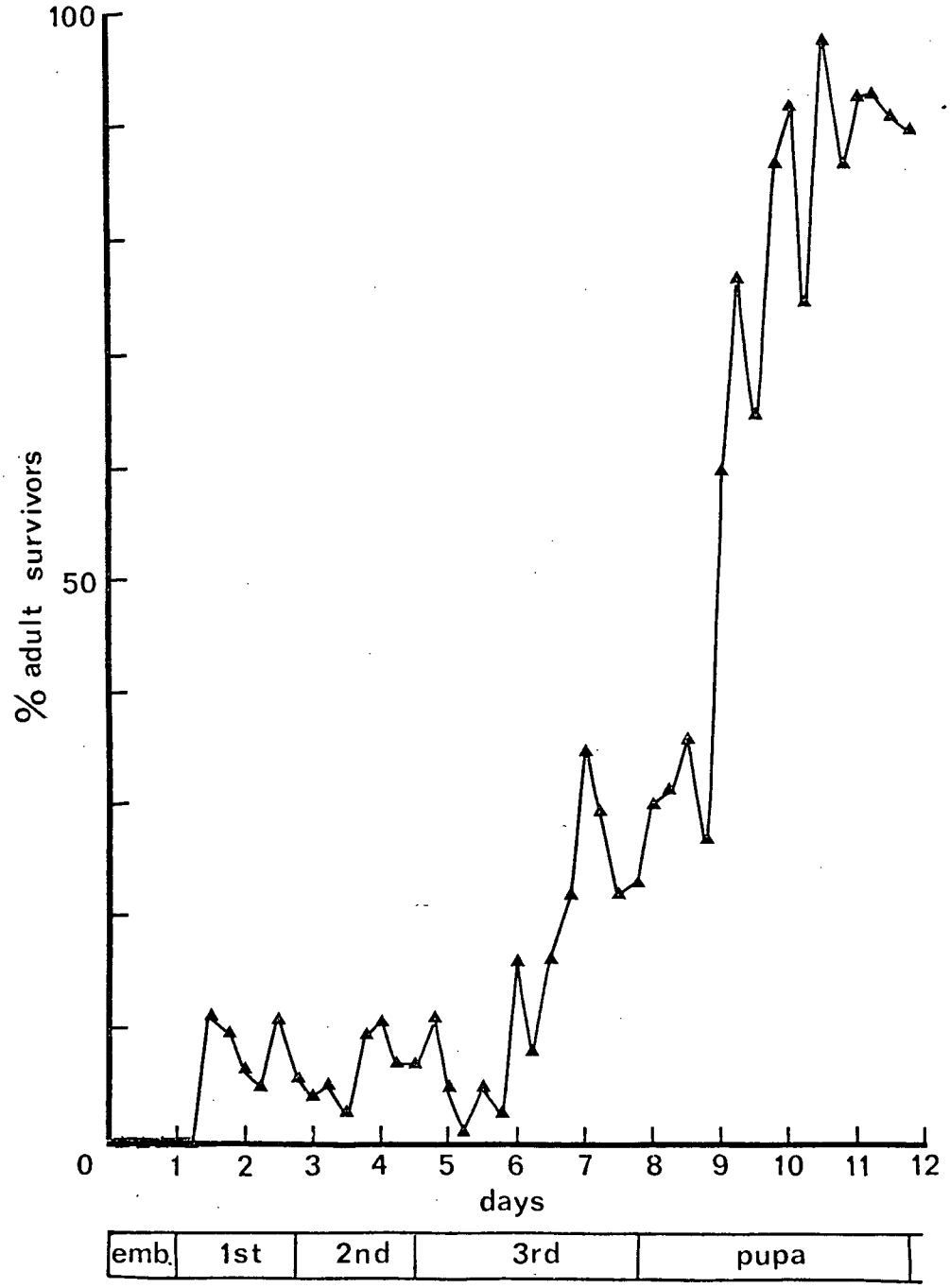

embryo
1st instar
100
50
% hatched eggs
12
24
36
48
hours

the TSPs more precisely. Cultures synchronized to within 2 hours of deposition were established at 22°C and the vials were shifted to 29°C at successive 6-hour intervals (shift-up). Any shift-up at or before the 24 hour embryonic period significantly reduces the egg hatchability (Fig. 15). The reciprocal shift-down experiments were also performed by collecting eggs at 22°C, immediately transferring the cultures to 29°C and then shifting back down at 6-hour intervals. The only culture in which eggs hatched (9%) was shifted down at 6 hours. In all other cultures, no eggs hatched. These experiments corroborate the pulse experiments in defining an embryonic TSP for lethality.

The shift-ups also revealed a significant increase in survival to adulthood in cultures shifted up after 150 hours and an even more pronounced increase in shift ups after the white pre-pupae (wpp) stage at 216 hours (Fig. 16). Thus, the data indicate the existence of TSPs during the embryonic, mid-3rd instar and early pupal stages. In order to characterize the pupal TSP more fully, shifts were made at 6-hour intervals on cultures synchronized by collection of wpp at 22°C and 29°C.

Shift-up and shift-down experiments covering the 60-hour interval preceeding wpp formation were performed using 2 sets of cultures. One set was incubated at 22°C until wpp began to appear, then shifted up to 29°C. White pre-pupae were collected at 6 hour intervals and their incubation continued at 29°C for the shift-up experiment. The second set was shifted up to 29°C during the 3rd larval instar and incubated there until wpp began to appear. At this time, the cultures

Figure 16. Percentages of <u>add A</u>^{ts1} eggs which yield adults after successive shift-ups administered at different times during development. The developmental stages at 22°C at the time of shift-ups are shown at the bottom.


100
50
0
% adult survivors
1
2
3
4
5
6
7
8
9
10
11
12
days
emb.
1st
2nd
3rd
pupa

were shifted down to 22°C and wpp collected at 6-hour intervals for the shift-down experiments. For shift-ups and shift-downs covering the 60-hour interval after wpp formation, wpp were collected from cultures at 29°C and 22°C and shifted down and up respectively at 6-hour intervals. This technique of synchronizing the cultures at the wpp stage yields results which indicate a significant increase of adult survivors in those cultures shifted up at 6 hour or more after wpp formation (Fig. 17). This signals the end of a TSP. Similarly, decreases in adult eclosion begin to appear when shift-downs are made 6 hours or less before wpp formation (Fig. 17) and this indicates the beginning of a TSP. These experiments delineate a TSP extending from 6 hours before to 6 hours after wpp formation.

Twelve-hour heat and cold pulses were made straddling the wpp stage. The data clearly indicated that the frequencies of adult survivor increase when the cultures develop at permissive temperatures during the period 12 hours before and after wpp formation (Fig. 18). Data for cold pulses administered at 42 hours or more after wpp formation are not shown because of too few observations to be significant, for example, a 50% survival in a cold pulse 54 to 66 hours after wpp formation was based on 2 survivors out of 4 wpp. Twelve-hour heat pulses beginning as early as 54 hours before wpp formation were found to reduce the fraction of eclosing adults, while the same heat pulses 12 hours after wpp formation had no effect on adult eclosion (Fig. 18). The most severe reduction of adults was observed when the heat pulses were

Figure 17. Percentage of <u>add A</u>^{ts1} white pre-pupae (wpp) which eclosed as adults, △—△, after successive shift-ups and, ▽—▽, after successive shift-downs administered prior to and after wpp formation.

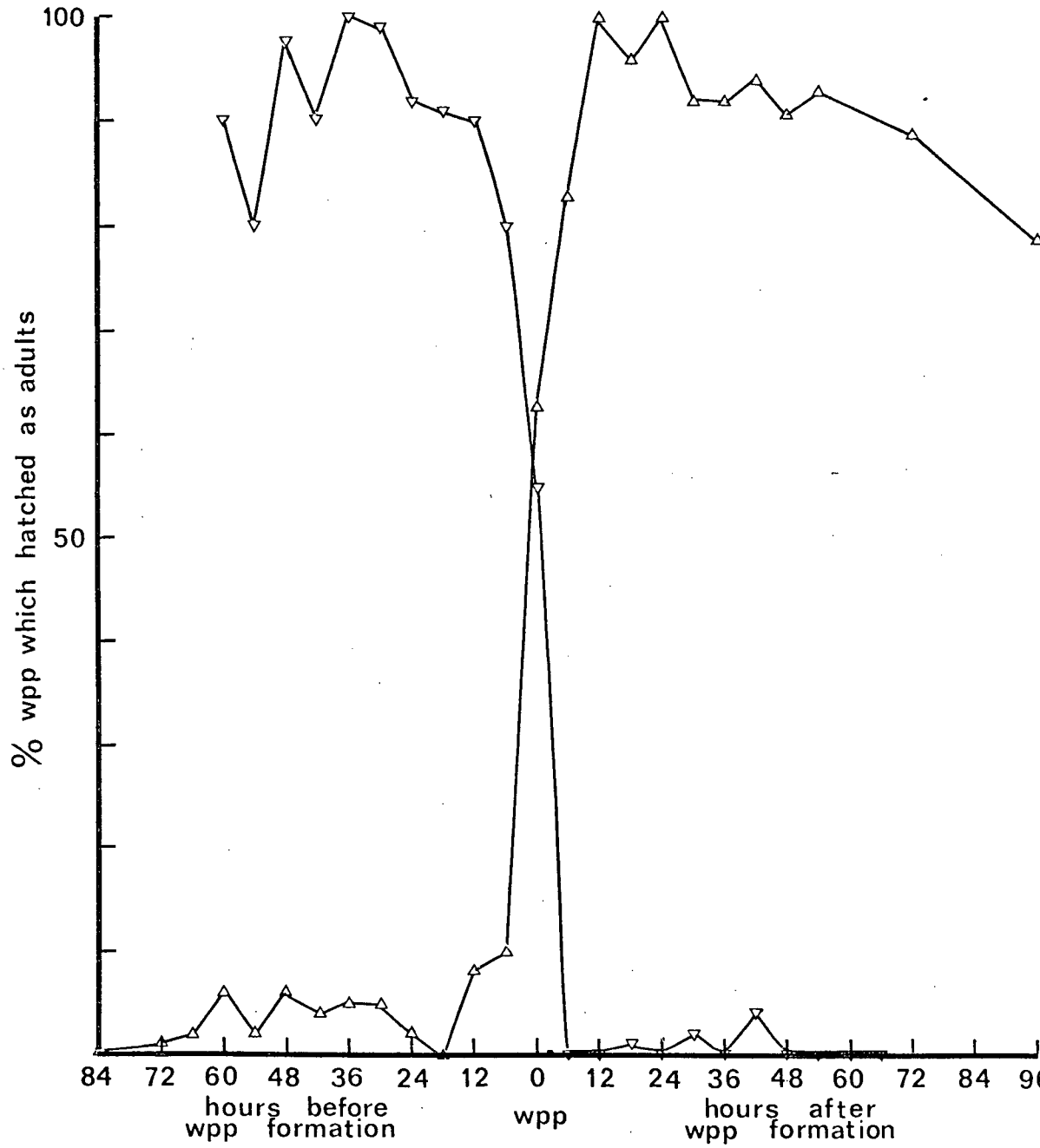
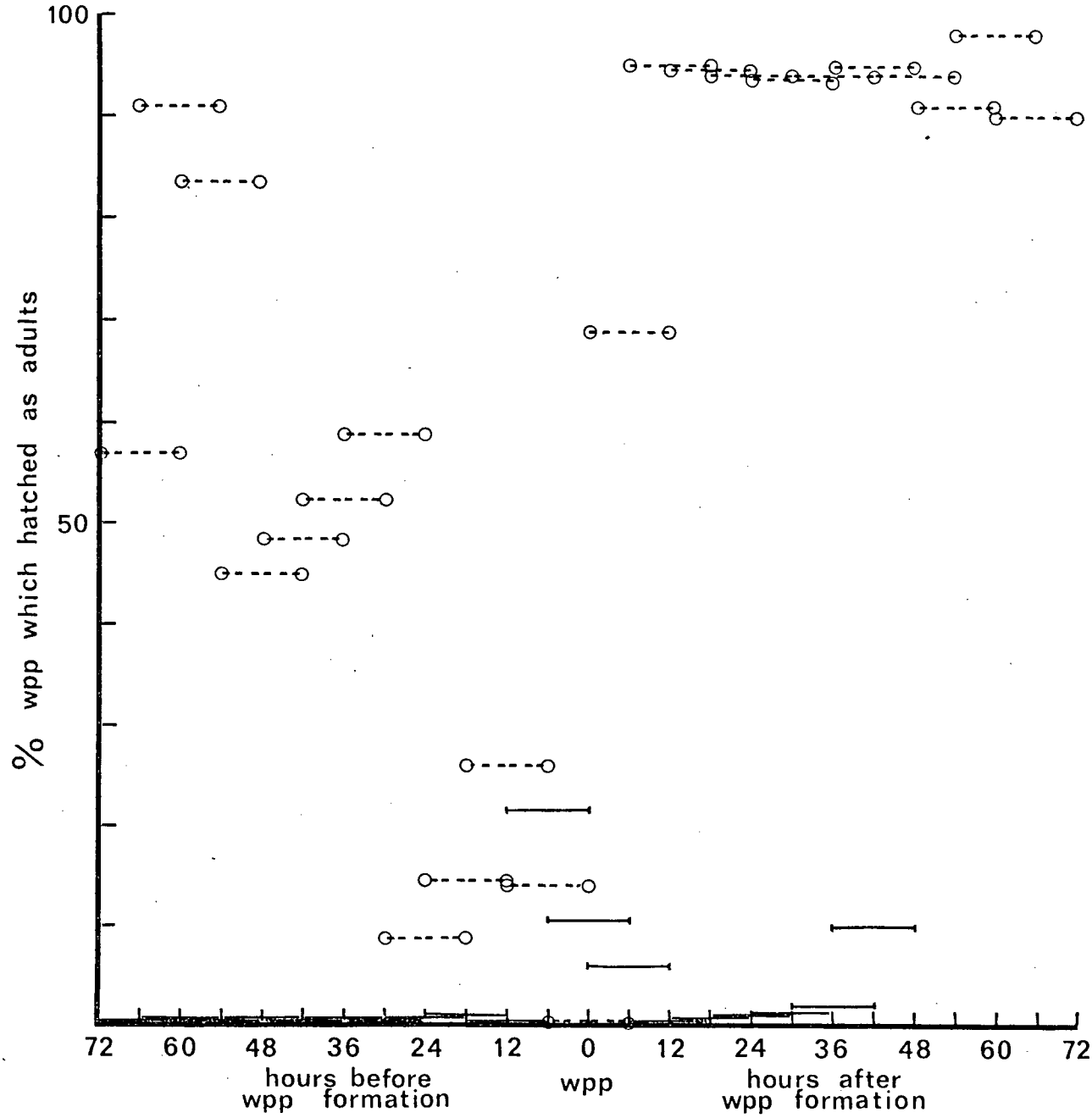

100
50
% wpp which hatched as adults
84 72 60 48 36 24 12 0 12 24 36 48 60 72 84 96
hours before wpp formation
wpp
hours after wpp formation

Figure 18. Percentage of add A^{ts1} white pre-pupae (wpp) which eclosed as adults, O----O, after 12 hour heat pulses (29°C) and, ⊢——⊣, after 12 hour cold pulses (22°C), administered prior to and after wpp formation.


100
50
% wpp which hatched as adults
72
60
48
36
24
12
0
12
24
36
48
60
72
hours before wpp formation
wpp
hours after wpp formation

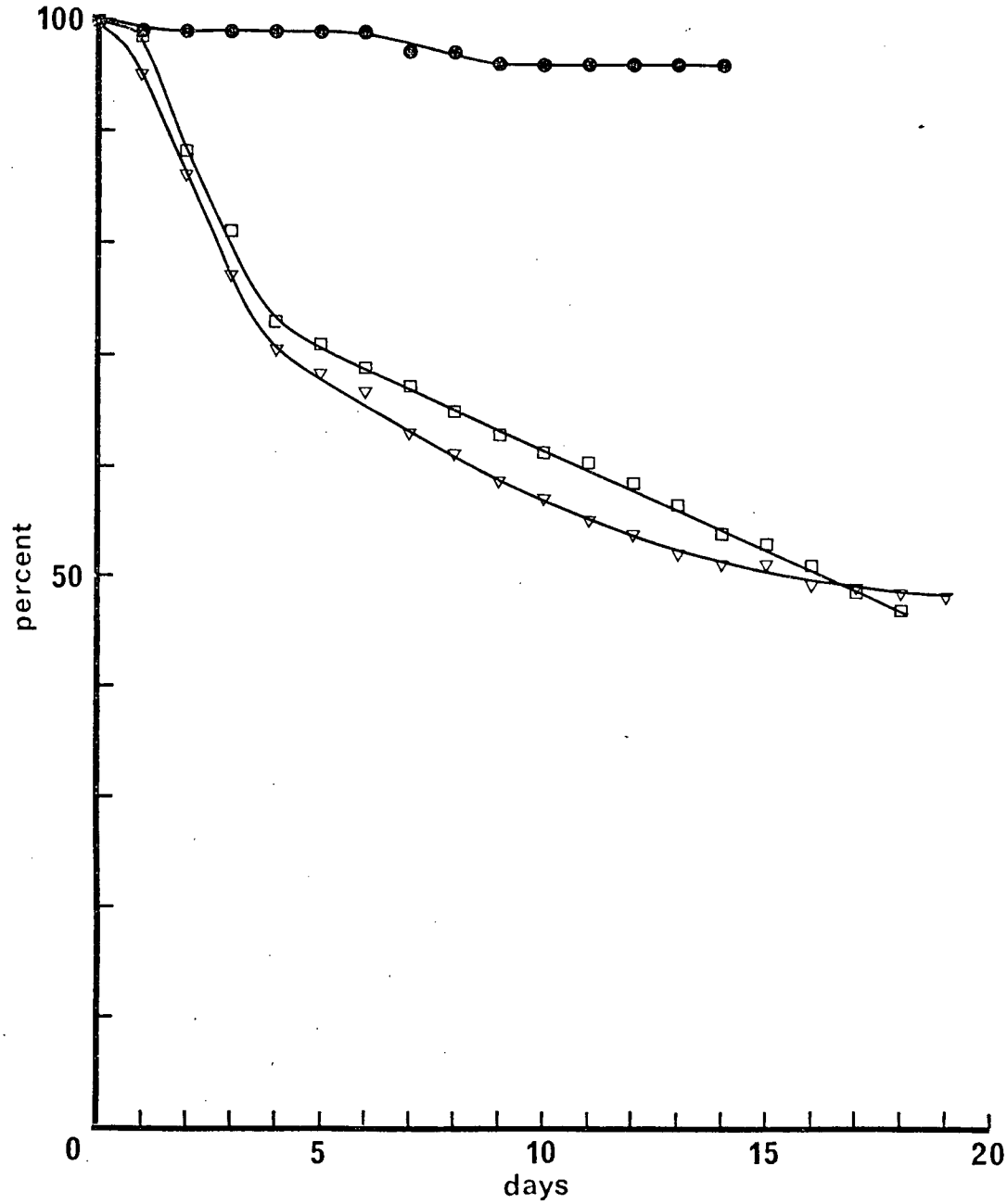
performed in the interval from 30 hours preceeding wpp formation to 6 hours following wpp formation.

Although there are minor discrepancies between the shift and pulse experiments defining the start of the TSP, these experiments indicate a TSP around the wpp stage which commences at least 6 hours prior to and ends 6 hours following wpp formation. Thus, we have shown that the add A^{ts1} mutant has 3 TSPs; one embryonic TSP which occupies the whole embryonic period of 24 hours, one 12 hour TSP which begins at 6 hours before wpp formation and ends at 6 hours after wpp formation, and another in the adult stage.

Genetic Mosaic Studies: The 292 mosaics used in this analysis were recovered from the cross y w add A^{ts1}/Y males X In(1)w^{vC}/v w spl females. The mosaics were developed and mosaic patches were identified at 22°C. All mosaics were shifted to 29°C within 2 days of eclosion and observed for 19 days for drop-dead behaviour.

It was found that survival (or lethality) of the add A^{ts1} mosaics shifted to 29°C do not show any signs of levelling off during the 19 days of observation (Fig. 19). Therefore, it is not possible to assign a drop-dead phenotype to these mosaics due to the possibility that not all of them with a premature ts lethal phenotype were scored. Fortunately, each mosaic was also observed for the onset of behavioural abnormalities. It was noted that some mosaics demonstrated individual leg paralysis beginning the first day after the shift up to 29°C (Fig. 19). In most cases, the leg paralysis phenotype was observed at least

Figure 19. Percentage of, □, 292 add A^{ts1} mosaic survivors at 29°C; ●, 75 y w add A^{+} mosaic survivors at 29°C (control); and △, % plot of 292 X 6 = 1752 legs for non-paralysis.


100
50
0
percent
5
10
15
20
days

two days before lethality. The major exceptions were those mosaics dead on day two and a fraction of those dying on day two. Unfortunately, no observations were made before day one. It was noted, however, that all six legs of many mosaics dying on days two and three had become severely paralyzed the previous day. Thus, in plotting the data for leg paralysis (Fig. 19), it was assumed that those mosaics which died during the first three days, but had not shown signs of motor paralysis, were mutant for all leg foci. This assumption seems reasonable for the following reasons: (1) In those mosaics which did show leg paralysis and early death, all six legs were paralyzed. (2) In general, there is a good correlation between time of death and the number of paralyzed legs. (3) Very few y w add A^{ts1} males which survived incubation at 29°C for several days, died before showing any motor debilitation. (4) Mosaics dying early were generally mutant for cuticle in the lower thorax, tissue nearest the add A^{ts1} foci as determined below. (5) Few control, y w add A^{ts1+}, mosaics died during the first 14 days of incubation at 29°C.

If the number of non-paralyzed legs were plotted against the days of observation (Fig. 19), it was found that the curve levels off at the 50% mark at about day 16. This is consistent with the interpretation that the behaviour of each leg is controlled by a single focus, and the chance that such a focus will be mutant is, like any cuticle marker, 50%. Thus, it seems justifiable to classify those legs that were paralyzed on day 0 to day 16 inclusive as mutant and

the remaining legs normal.

Fate mapping analysis showed that the primary foci for leg paralysis lies in the mesodermal tissues of the blastoderm. Each leg has its own independent focus located at about 22 sturts from its respective coxa (Fig. 20).

Drug Studies: In order to test whether synaptic functions are involved in the drop-dead mutations, the mutants were exposed to varying concentration of different psychotropic and neurotropic drugs known to affect neural functions in mammals. The results of the drug studies are summarized in Table 9. Complete results of the drug studies are presented in the Appendix.

add A^{ts1} is more sensitive to Yohimbine, an adrenergic blocker, and more resistant to pilocarpine nitrate which is an agonist of cholinergic system.

Photomicrographic Studies: Because add A^{ts1} mutants have an embryonic TSP, the embryonic development of add A^{ts1} and Oregon-R wild type were compared and examined for abnormalities using photomicrography.

The results of photomicrography of embryos kept at 29°C for varying lengths of time can be seen in Fig. 21. The pictures with a darker background were taken using a polarizer while those with a brighter background were taken using standard bright field illumination. The malpighian tubules and muscles will be birefringent under polarized light to provide an easier way of identifying these

Figure 20. Fate map of add A^{tsl}. a, antenna; anp, anterior notopleural bristle; hu, humerus; oc, ocellar; 1c, coxa 1; 2c, coxa 2; 3c, coxa 3; 3s, 3rd sternite. ●, foci of add A^{tsl}.

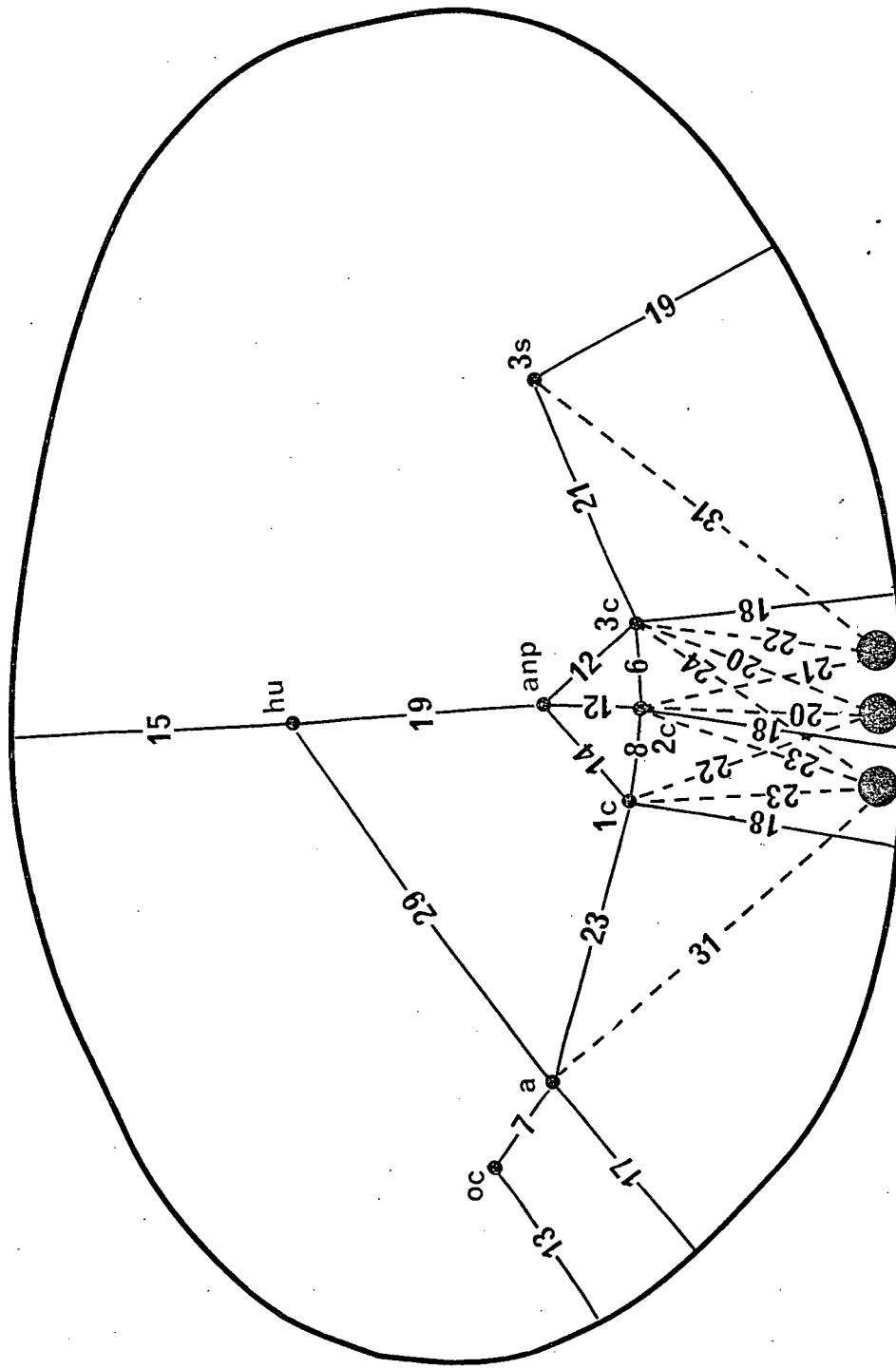
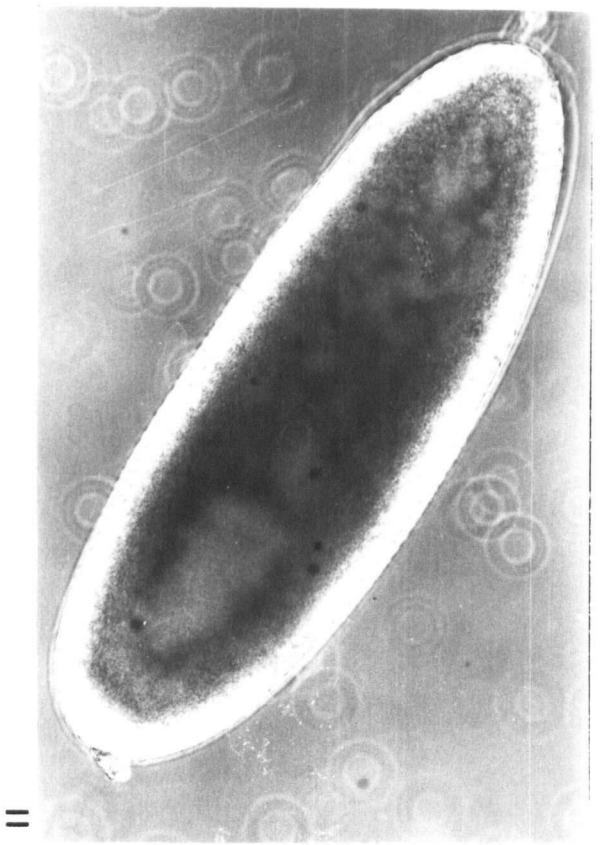

3s
19
21
31
3c
18
22
21
20
24
12
6
anp
12
20
18
15
hu
19
2c
8
14
23
22
23
1c
18
29
23
31
a
7
oc
17
13

Table 9

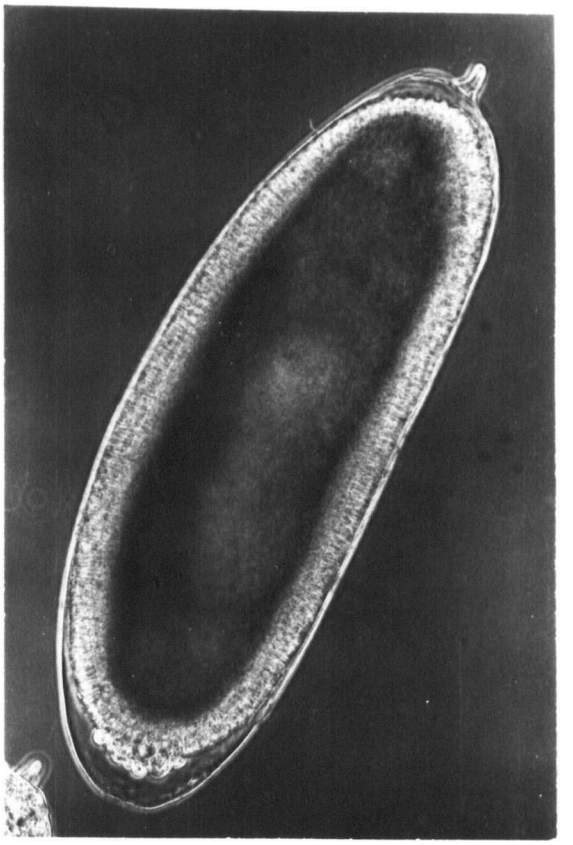
List of drugs which have a more pronounced effect on the viability of two drop-dead strains. The effects of the same drug on Oregon-R are also shown. Drugs were dissolved in water to make different concentrations before they were added to the instant Drosophila medium (Boreal). Cultures with no drugs dissolved in the water serve as controls.

		Ore-R		add A^{ts1}		ses E^{1}	
		pupae / larvae %	adults / pupae %	pupae / larvae %	adults / pupae %	pupae / larvae %	adults / pupae %
DRUG	CONC. mg/ml						
Pilo-	.2	72.2	66.7	79.5	54.8		
carpine	.4	62.5	100.0	-	-		
nitrate	.8	60.0	100.0	71.1	88.9		
	1.6	0.0	-	56.4	81.8		
	3.2	0.0	-	63.4	65.4		
Carbamyl-	.75	73.8	96.8			82.1	91.3
choline	1.5	67.4	100.0			69.0	100.0
chloride	3.0	33.3	100.0			89.3	100.0
	6.0	0.0	-			60.7	70.0
	9.0	0.0	-			70.0	100.0
Yohimbine	.5	88.5	100.0	16.2	33.3		
	1.0	54.1	100.0	11.4	100.0		
	2.0	51.4	68.4	-	-		
	4.0	0.0	-	-	-		
	8.0	0.0	-	-	-		
H_2O		87.2	91.2	58.3	76.2	100.0	82.5
control		72.1	90.3	88.2	92.4	57.1	91.7
no drugs		82.5	100.0	44.7	94.1	96.4	92.6
		50.0	84.0	51.4	100.0	91.6	90.9

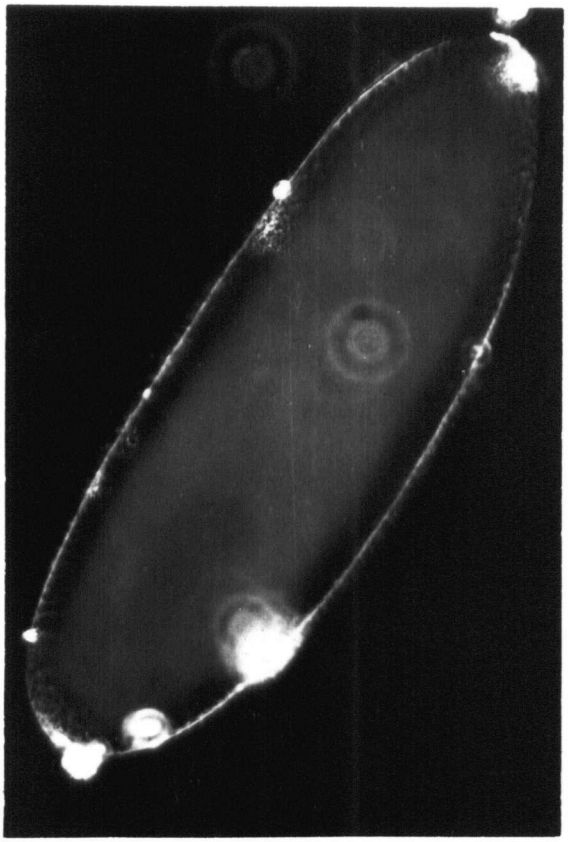
Figure 21. Photomicrographs of add A^{tsl} and Oregon-R embryos, developed at 29°C, at 2 hours post-fertilization: add A^{tsl} embryos under I, polarized light; II, bright field illumination; Oregon-R embryos under III, polarized light; IV, bright field illumination.



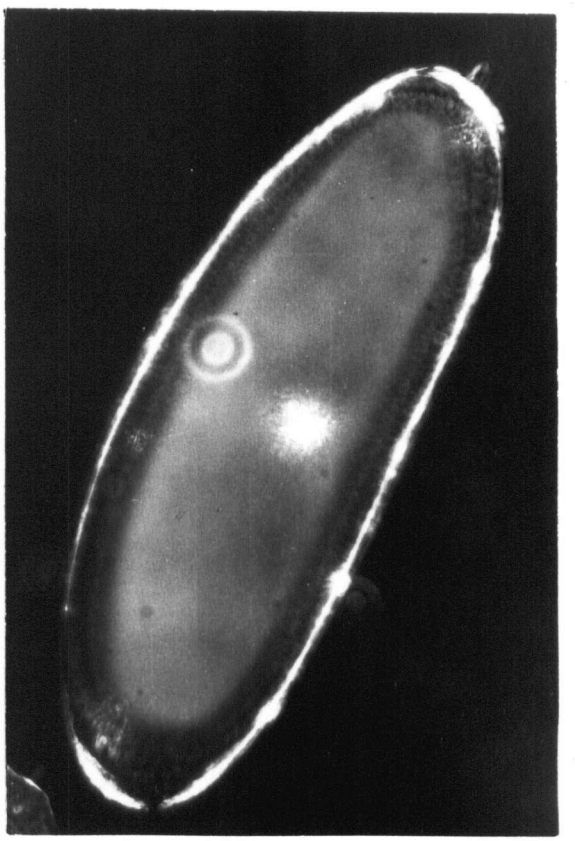
II



IV



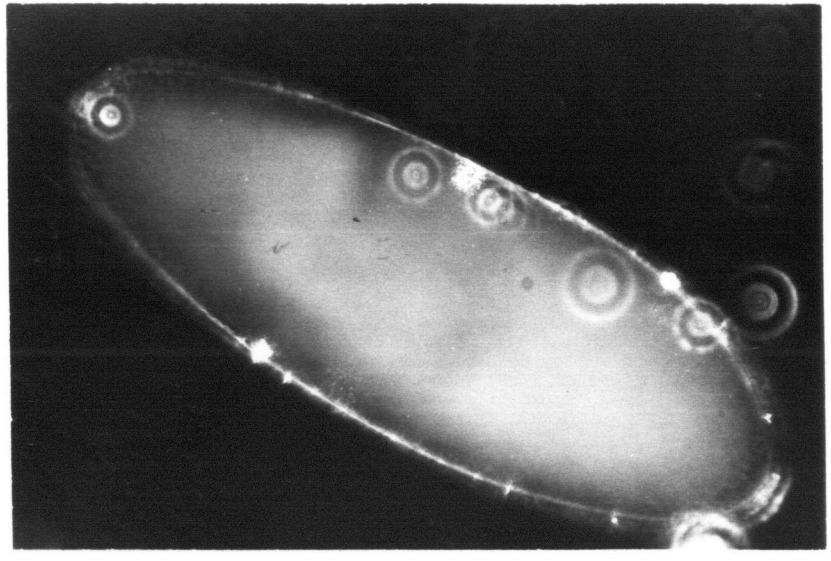
I



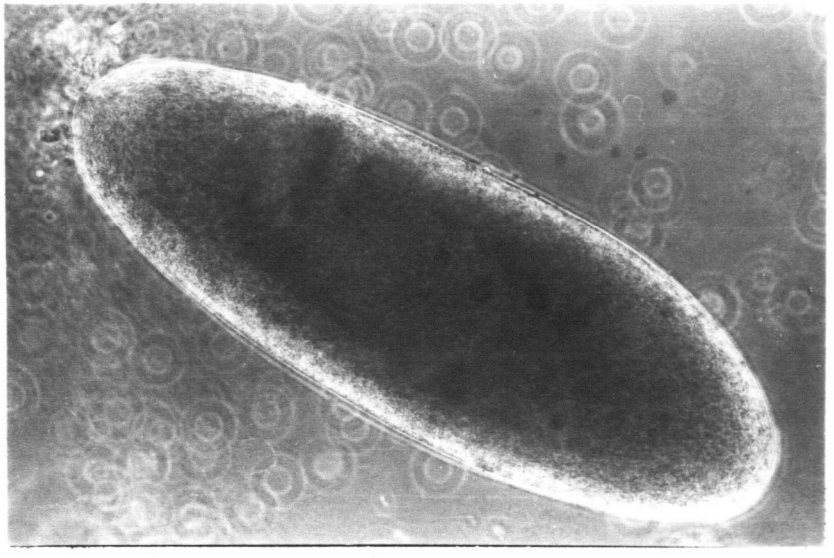
III

Figure 21 (cont.). Photomicrographs of add A^{ts1} and Oregon-R embryos, developed at 29°C, at 4 hours post-fertilization: Oregon-R embryos under V, polarized light; VI, bright field illumination; add A^{ts1} embryos under VII, polarized light; VIII, bright field illumination.

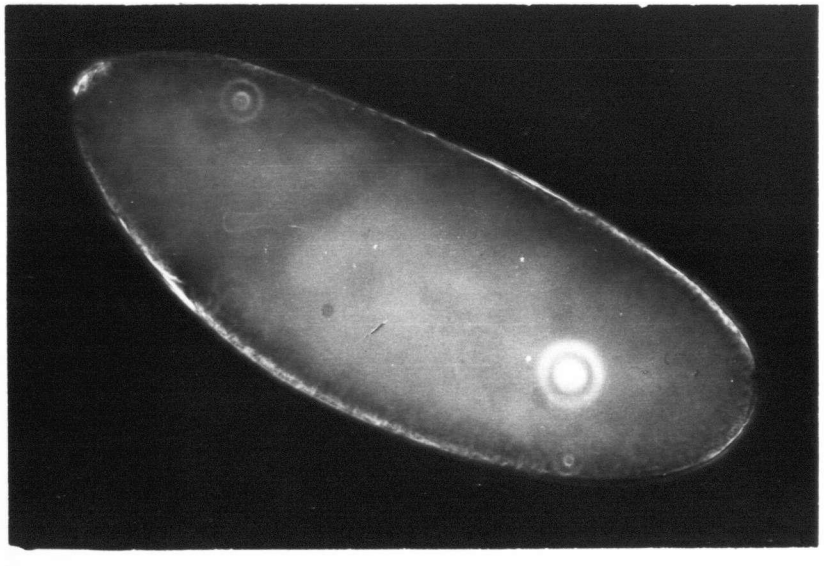
V



VI



VII



VIII

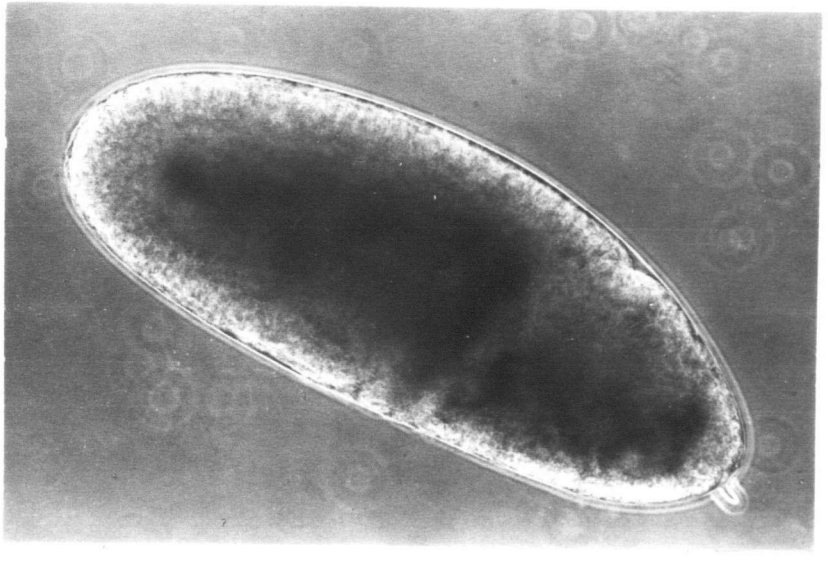
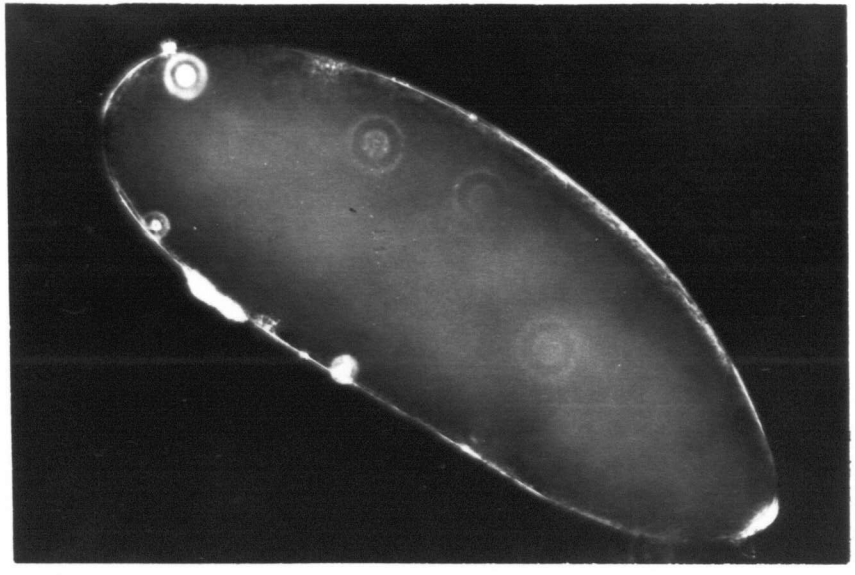
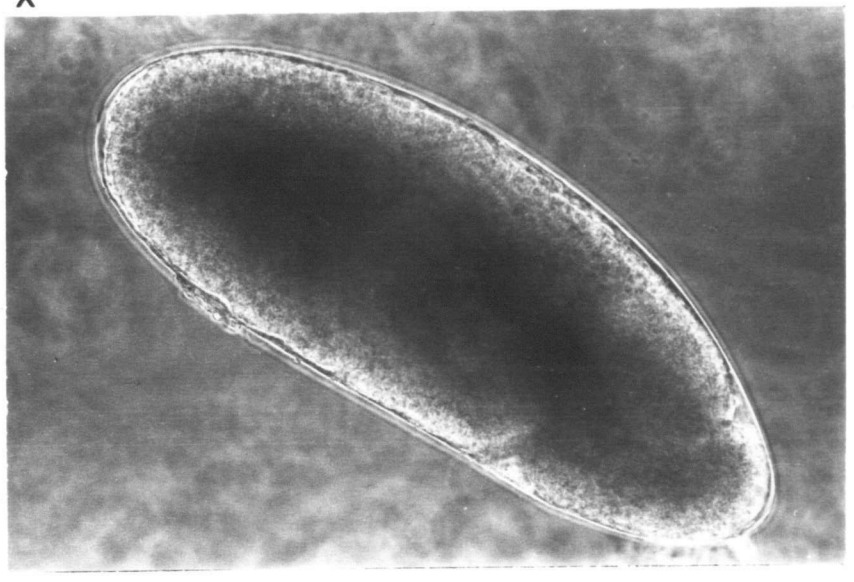


Figure 21 (cont.). Photomicrographs of *add A*^{ts1} and Oregon-R embryos, developed at 29°C, at 6 hours post-fertilization: *add A*^{ts1} embryos under IX, polarized light; X, bright field illumination; Oregon-R embryos under XI, polarized light; XII, bright field illumination.

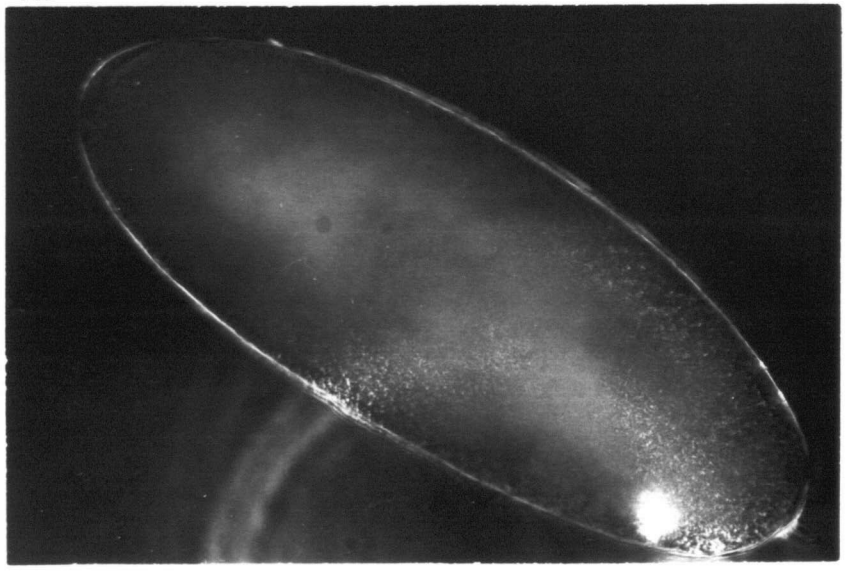
IX



X



XI



XII

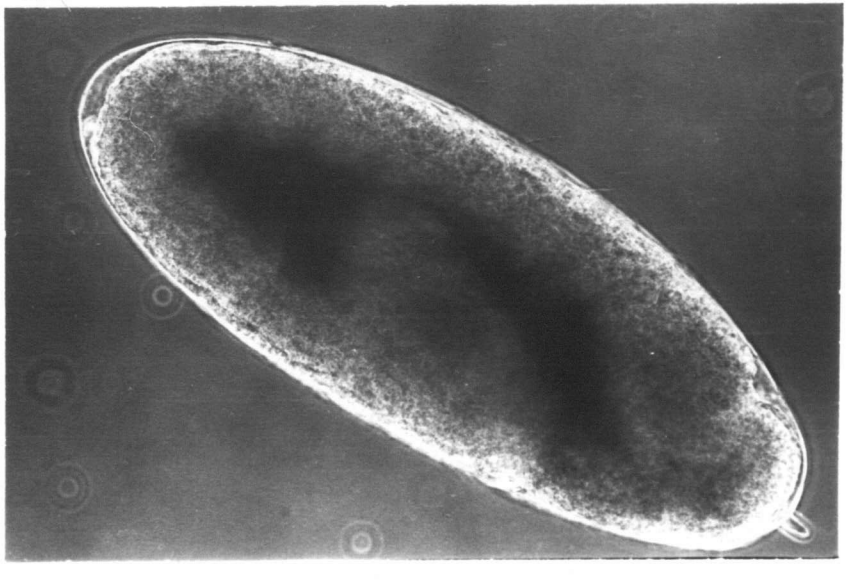
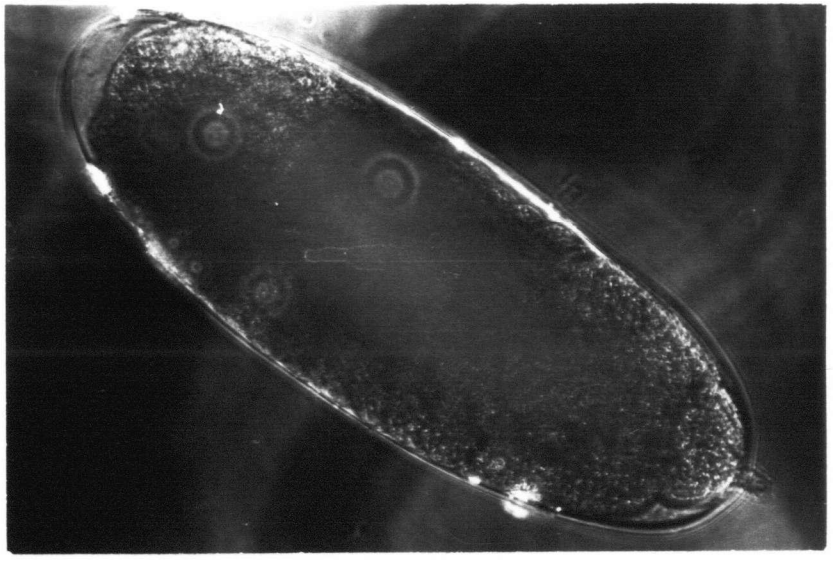
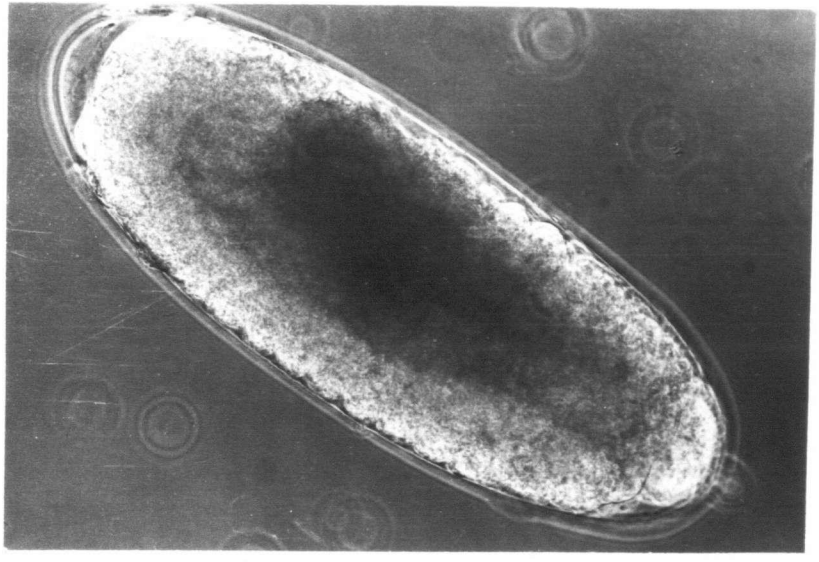


Figure 21 (cont.). Photomicrographs of add A^{ts1} and Oregon-R embryos, developed at 29°C, at 8 hours post-fertilization: add A^{ts1} embryos under XIII, polarized light; XIV, bright field illumination; Oregon-R embryos under XV, polarized light; XVI, bright field illumination.

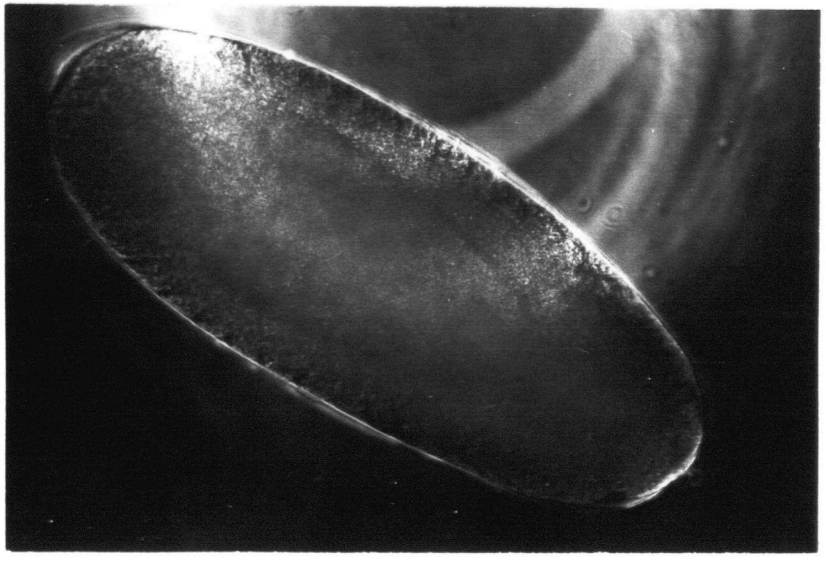
XIII



XIV



XV



XVI

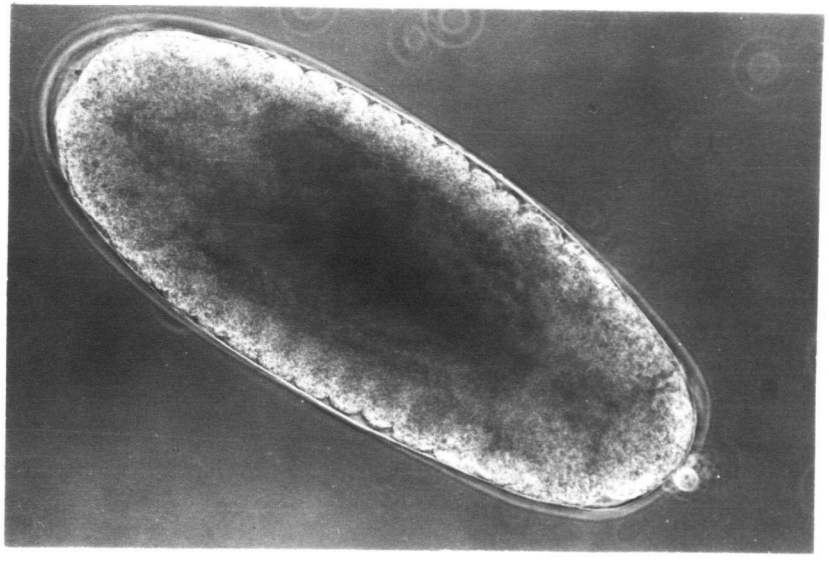
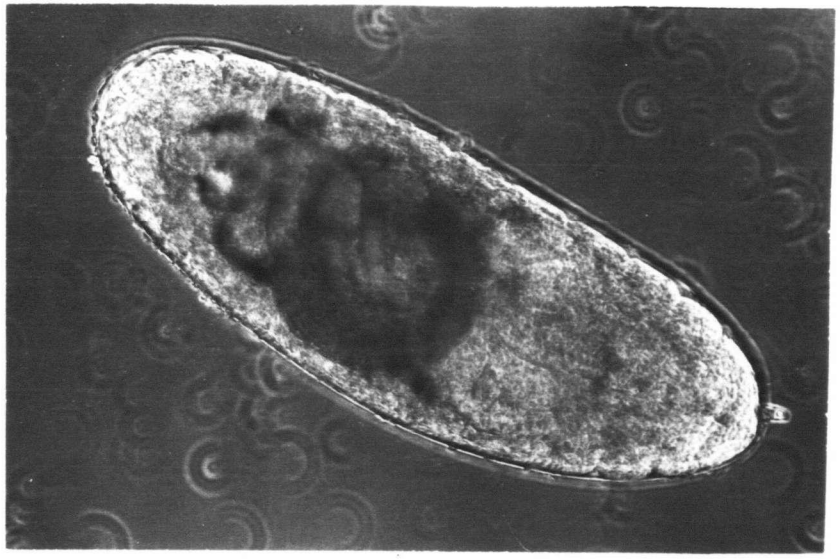


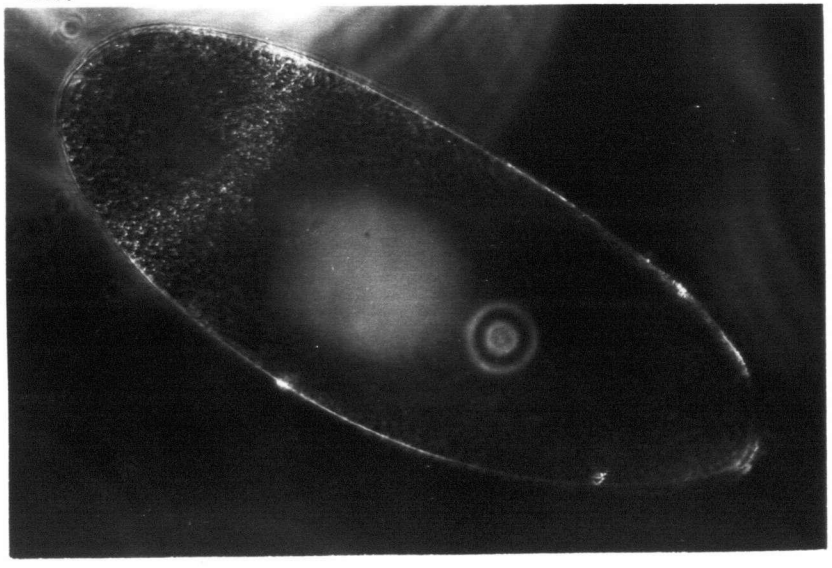
Figure 21 (cont.). Photomicrographs of add A^{ts1} and Oregon-R embryos, developed at 29°C, at 10 hours post-fertilization: add A^{ts1} embryos under xvii, polarized light; xviii, bright field illumination; Oregon-R embryos under xix, polarized light; xx, bright field illumination.

xvii

xviii



xix



xx

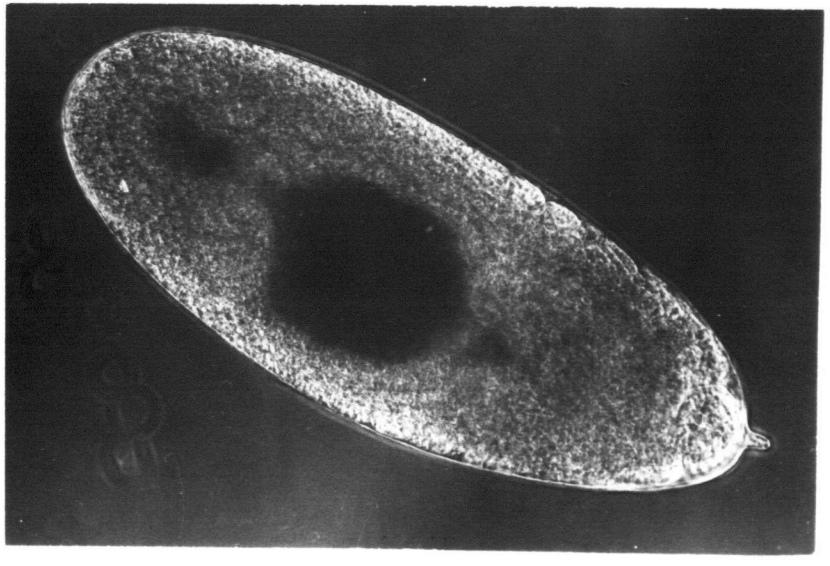
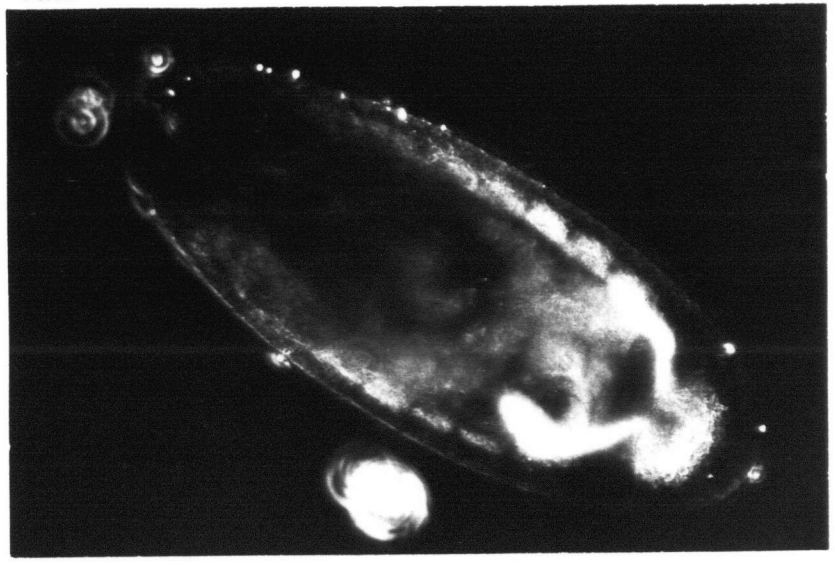
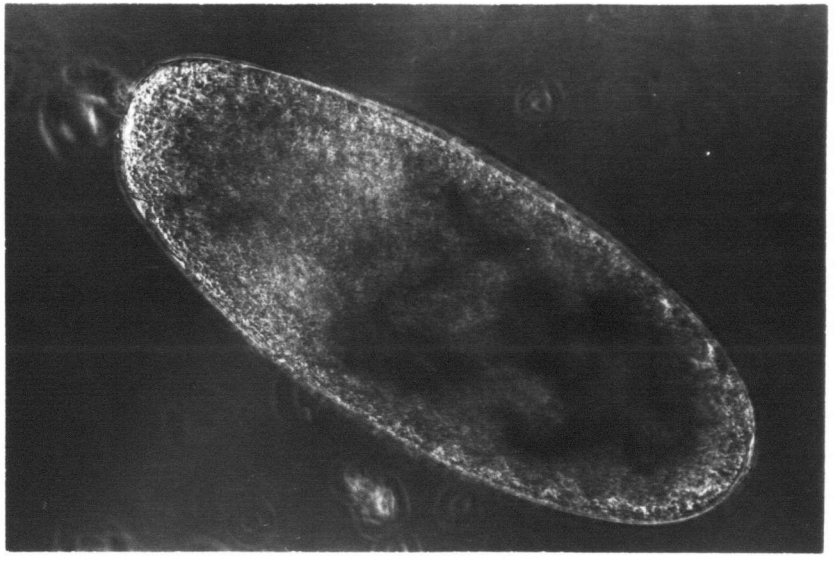


Figure 21 (cont.). Photomicrographs of add A^{ts1} and Oregon-R embryos, developed at 29°C, at 12 hours post-fertilization: add A^{ts1} embryos under XXI, polarized light; XXII, bright field illumination; Oregon-R embryos under XXIII, polarized light; XXIV, bright field illumination.

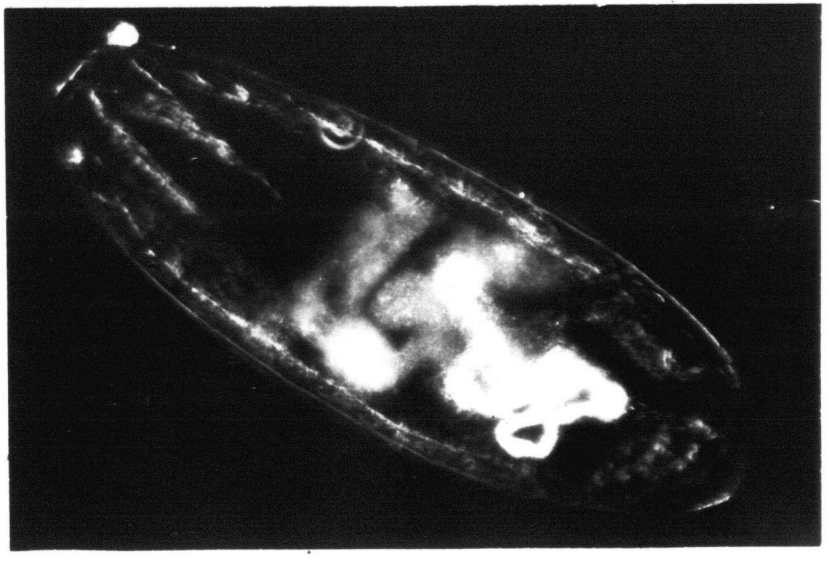
XXI



XXII



XXIII



XXIV

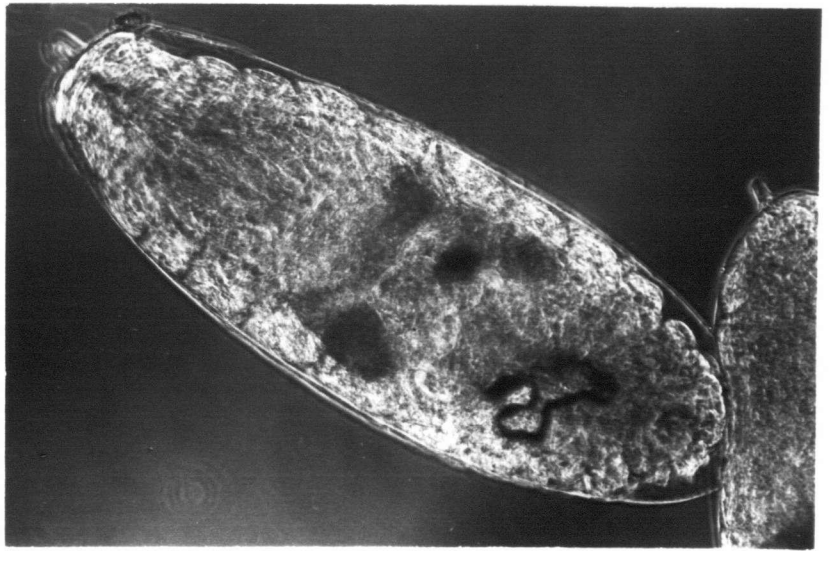
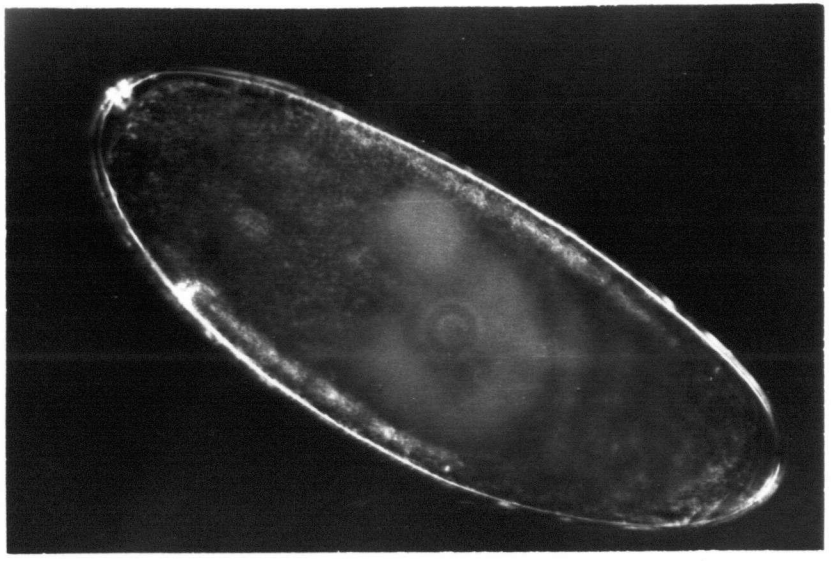
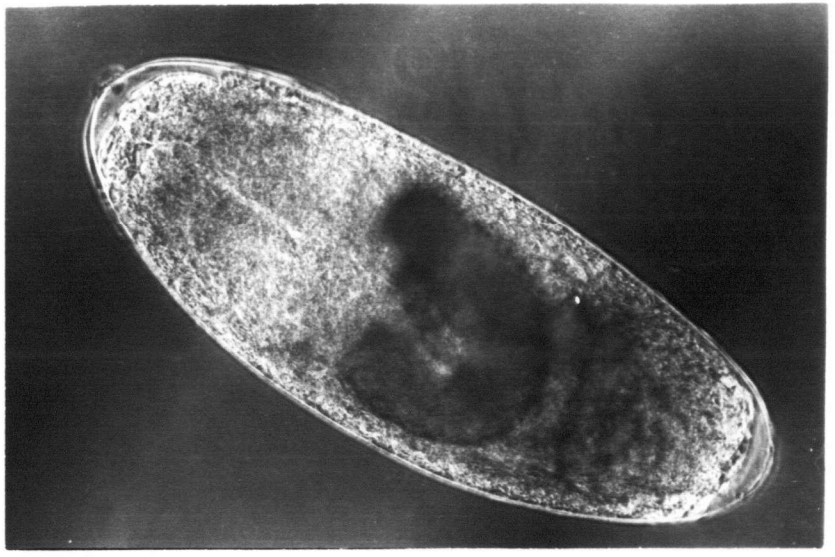


Figure 21 (cont.). Photomicrographs of *add A*^{ts1} and Oregon-R embryos, developed at 29°C, at 14 hours post-fertilization: *add A*^{ts1} embryos under XXV, polarized light; XXVI bright field illumination Oregon-R embryos under XXVII, polarized light; XXVIII, bright field illumination. mu, muscles; ma, malpighian tubules.

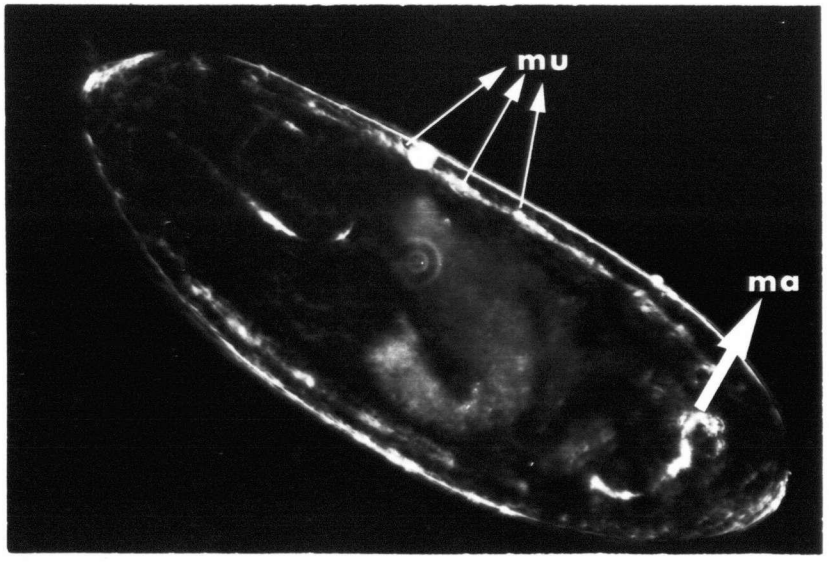
XXV



XXVI



XXVII



XXVIII

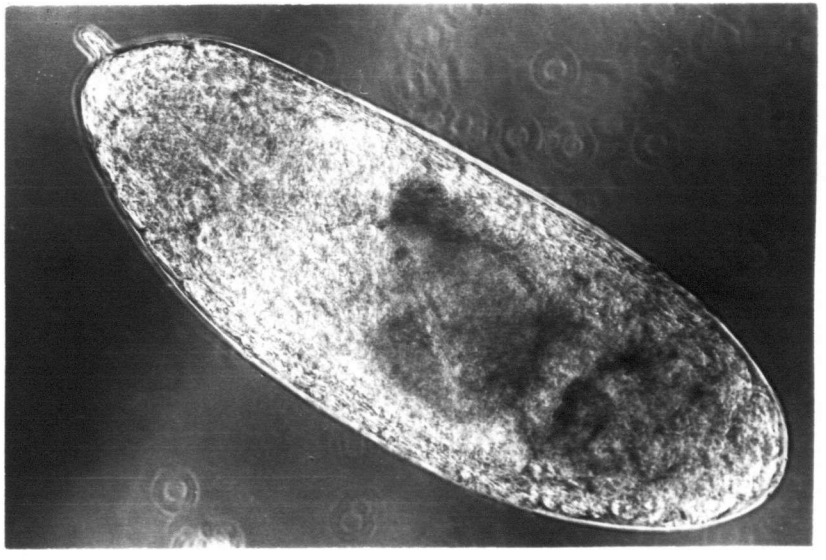
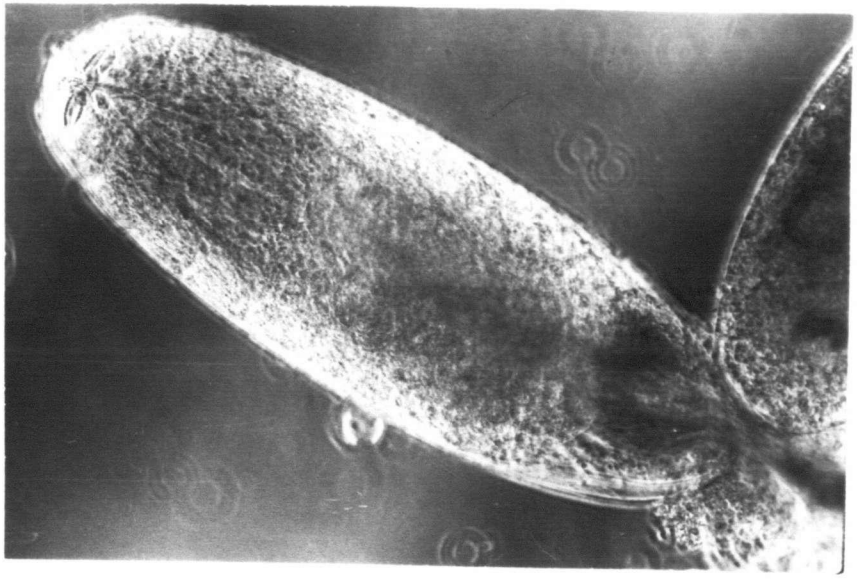


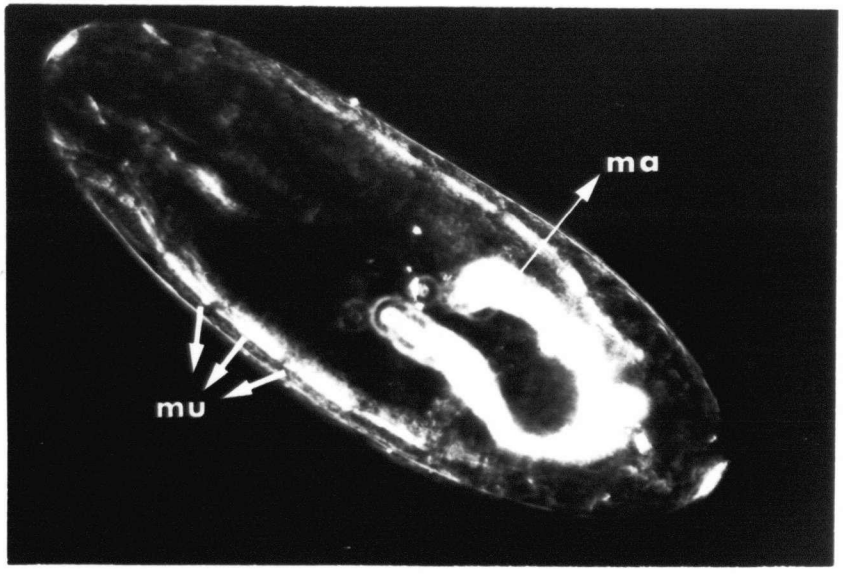
Figure 21 (cont.). Photomicrographs of add A^{ts1} and Oregon-R embryos, developed at 29°C, at 16 hours post-fertilization: add A^{ts1} embryos under XXIX, polarized light; XXX, bright field illumination; Oregon-R embryos under xxxi, polarized light; xxxii, bright field illumination. mu, muscles; ma, malpighian tubules.

XXIX

XXX



xxxi



xxxii

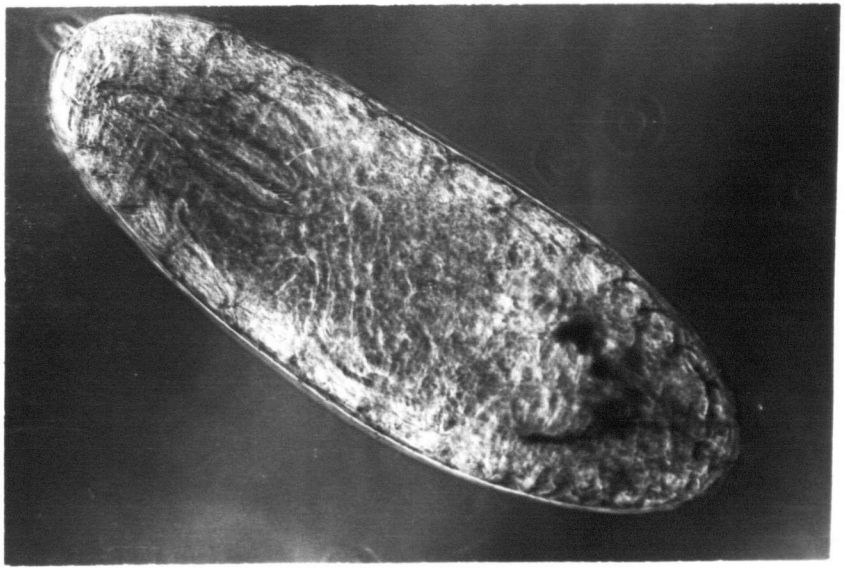


Figure 21 (cont.). Photomicrographs of add A^{ts1} and Oregon-R embryos developed at 29°C, at 17½ hours post-fertilization: add A^{ts1} embryos under xxxiii, polarized light; xxxiv, bright field illumination; Oregon-R embryos under xxxv, polarized light; xxxvi, bright field illumination.

xxxiii

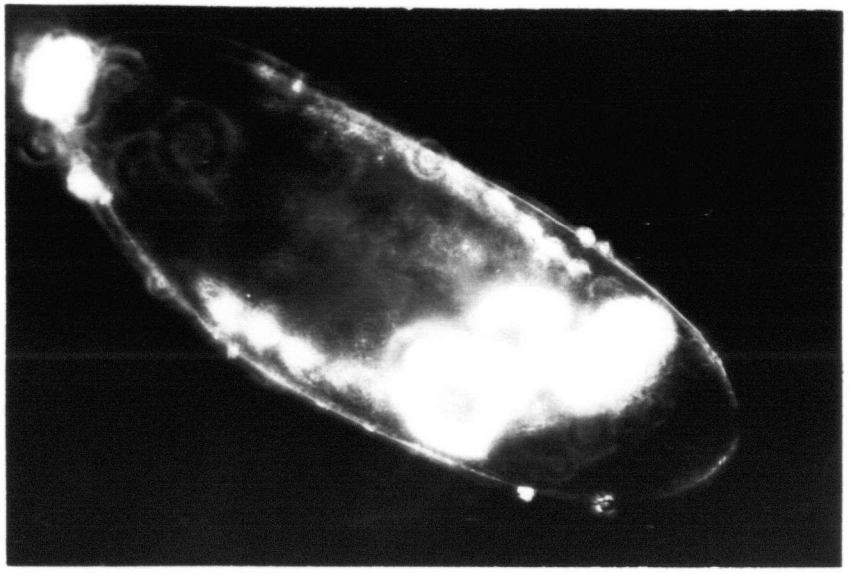
xxxiv

xxxv

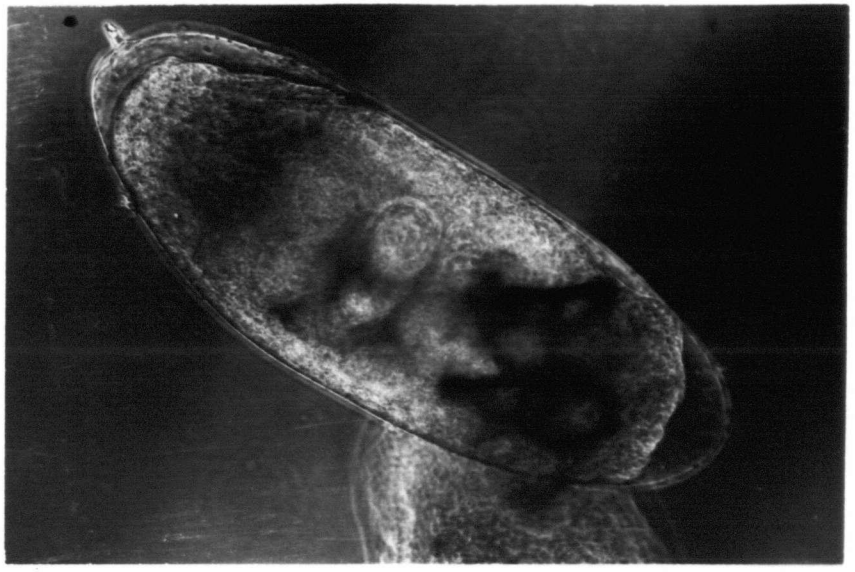
xxxvi

Figure 21 (cont.). Photomicrographs of add A^{ts1} embryos, developed at 29°C: at 22 hours post-fertilization under xxxvii, polarized light; xxxviii, bright field illumination; at 46 hours post-fertilization under xxxix, polarized light; xl, bright field illumination.

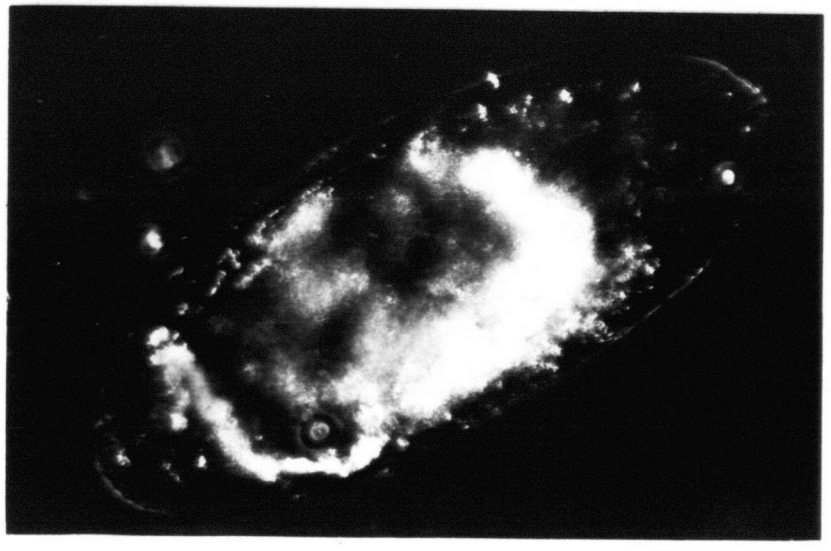
xxxvii



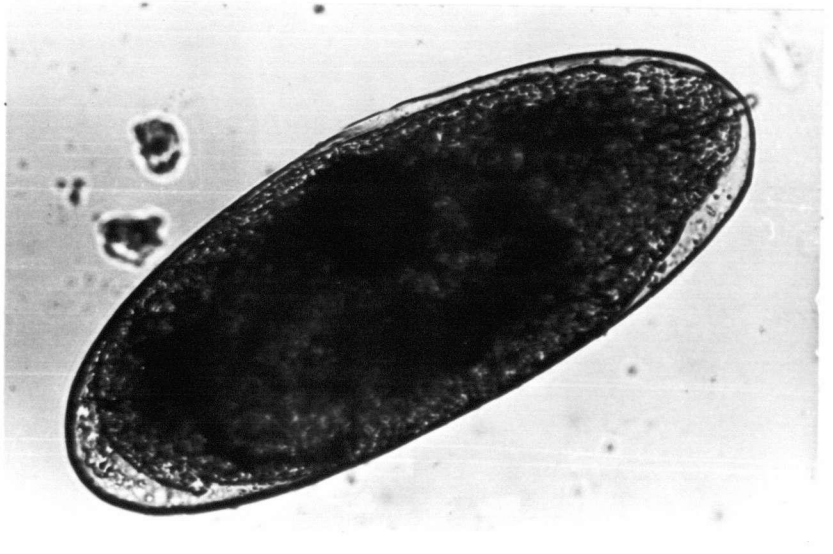
xxxviii



xxxix



xL



structures in the developing embryos.

From fertilization (0 hour) through gastrulation (3 - $3\frac{1}{2}$ hour) and head segmentation (6 hour) to dorsal and ventral segmentation (8 hour), there were no detectable differences between the add A^{ts1} and Oregon-R embryos. However, after 10 hours at 29°C, the malpighian tubules and muscles of add A^{ts1} embryos appear to develop at a faster rate since Oregon-R have none at this time while the 22°C controls of both Oregon-R and add A^{ts1} embryos developed at exactly the same rate. It should be noted that the add A^{ts1} embryos may have an overall increase in rate of development of other tissues as well, but only the muscles and malpighian tubules were easily detected by the techniques used here. By 12 hours, the add A^{ts1} embryos have more prominent but poorly defined muscles and malpighian tubules and the segmentations once visible earlier have disappeared. In contrast, Oregon-R at this stage have well defined muscles and malpighian tubules. At 14 hours, Oregon-R embryos start to show advanced muscle development and segmentation while the add A^{ts1} embryos completely lack segmentation. At 16 hours, the muscles and malpighian tubules in the add A^{ts1} embryos become less organized and more dispersed while in the Oregon-R embryos well defined muscles form distinct bands along the longitudinal sides of the embryos. At this stage, the Oregon-R embryos also have trachae while add A^{ts1} have none. By $17\frac{1}{2}$ hours, when most Oregon-R embryos have hatched, the muscles and the malpighian tubules of the add A^{ts1} embryos, though disorganized, are still present but the embryos fail

to hatch. At 22 hours, these structures still persist in add A^{ts1} embryos but no larvae hatch.

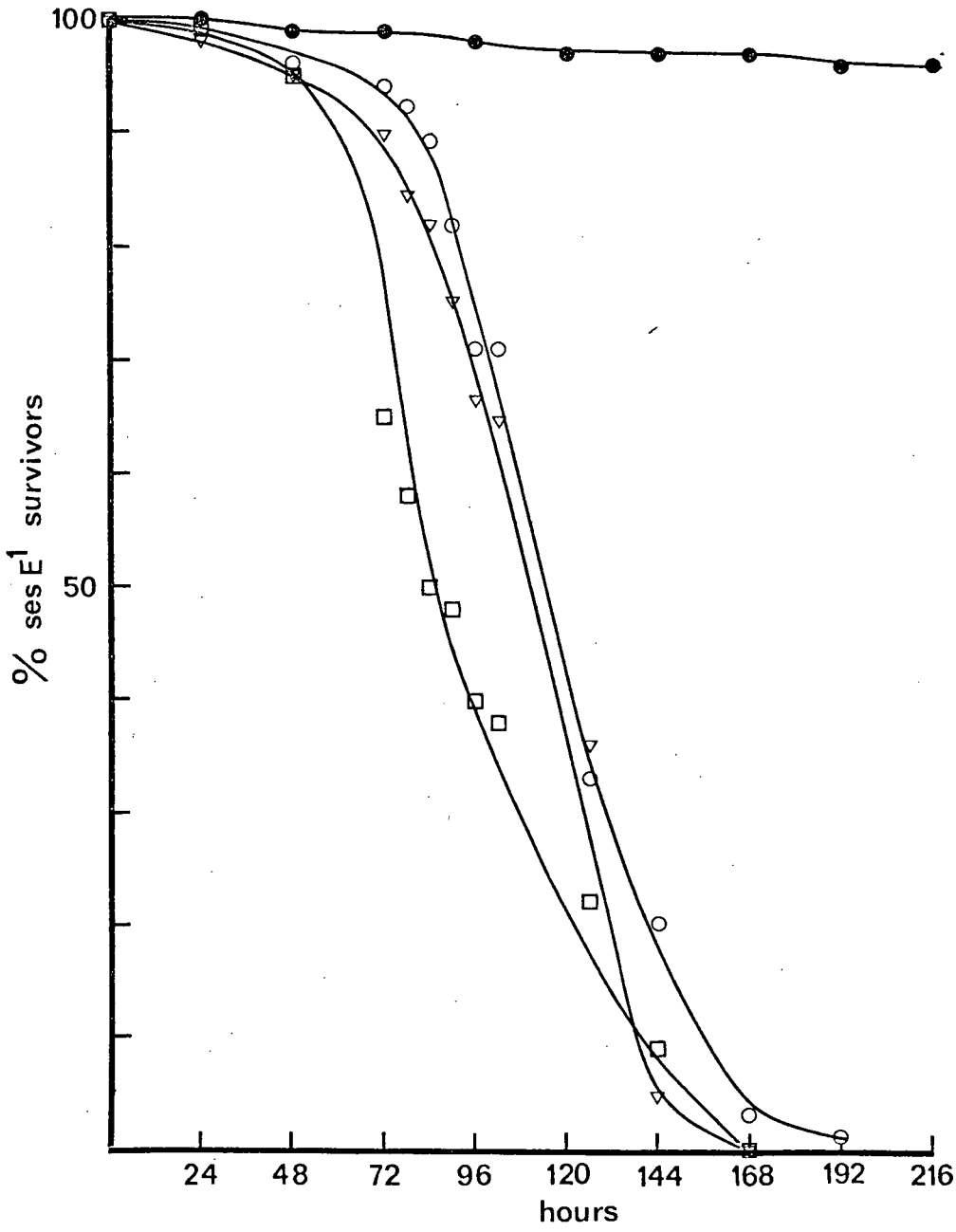
In summary, it appears that the muscles and malpighian tubules of add A^{ts1} embryos, although formed at an earlier stage than Oregon-R, are never well organized and are also ill defined when compared to Oregon-R embryos. Therefore, it seems that the lethality of add A^{ts1} at 29°C is not due to a lack of muscle or malpighian tubule development but to a failure of the muscular system and the malpighian tubules to maintain their integrity and to organized themselves. For example, the muscular sysstem fails to form distinct bands. It should be noted that the malpighian tubules and muscles were used as indicators of embryo development because of their ease of identification under polarized light. Moreover, these are very gross morphological differences as observed through the light microscope and the more subtle differences, if present, are beyond the capability of the methodology used here.

ses E^1

Characterization of adult drop-dead behaviour: When ses E^1 flies are shifted to 29°C, almost 95% die within 6 days (Fig. 22) whereas 95% of Oregon-R flies usrvive for at least 17 days at 29°C (Fig. 12).

Characterization of TSPs: The description of TSPs of ses E^1 has already been presented in Chapter 2. To recapitulate, ses E^1 flies have 2 TSPs - a 70-hour TSP extending from mid-1st instar larval to mid-2nd instar larval stage and a 90-hour TSP during the transition

Figure 22. Survival of ▽, 1 day old; ○, 5 day old; □, 10 day old ses E^1 adults at 29°C and , 1-5 day old ses E^1 adults at 22°C.

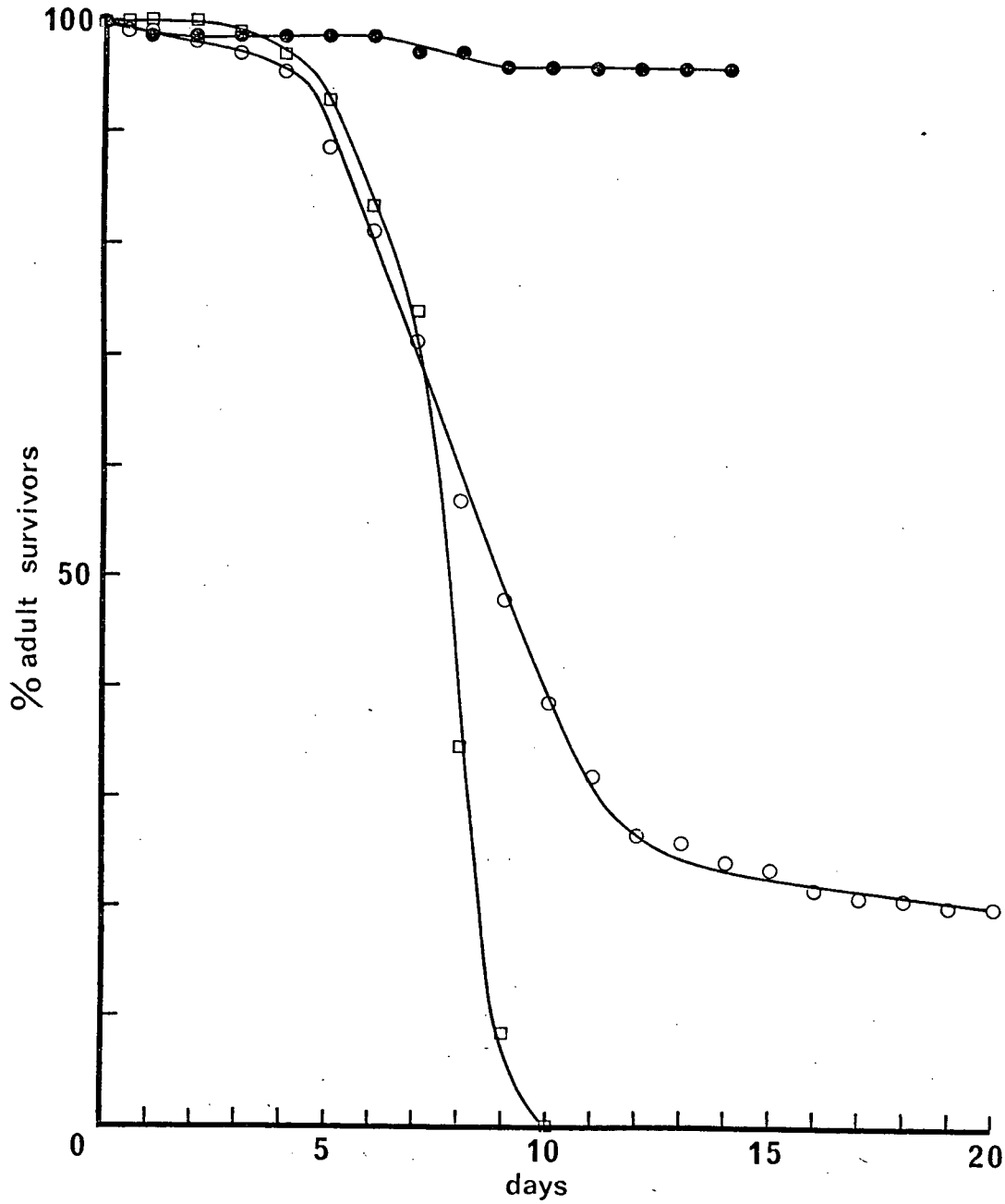

100
50
% ses E1 survivors
24
48
72
96
120
144
168
192
216
hours

of 3rd instar larval to pupal stage (Fig. 4, Fig. 5).

Genetic Mosaic Studies: 229 mosaics were recovered among offspring of the cross y w ses E^1/Y males X In(1)w^{vC}/y w spl females. The details of the mosaic analysis have already been mentioned in the previous chapter. The survival curve of the ses E^1 mosaics (Fig. 23) suggests that those mosaics which die on or before the 13th day are drop-dead in phenotype. This allows a more simple and straight forward fate mapping analysis. Control XO males recovered from the same cross, are a result of fertilization of a nullo-X egg by a y w ses E^1 sperm. These XO males all die before day 10. It was found that the embryonic focus of ses E^1 is located in the area of neuroblasts about 9 sturts below coxa 2 and coxa 3 in the blastoderm fate map (Fig. 10, Chapter 2).

Drug Studies: ses E^1 is more resistant than Oregon-R to carbamylcholine chloride, an agonist of cholinergic system (Table 9).

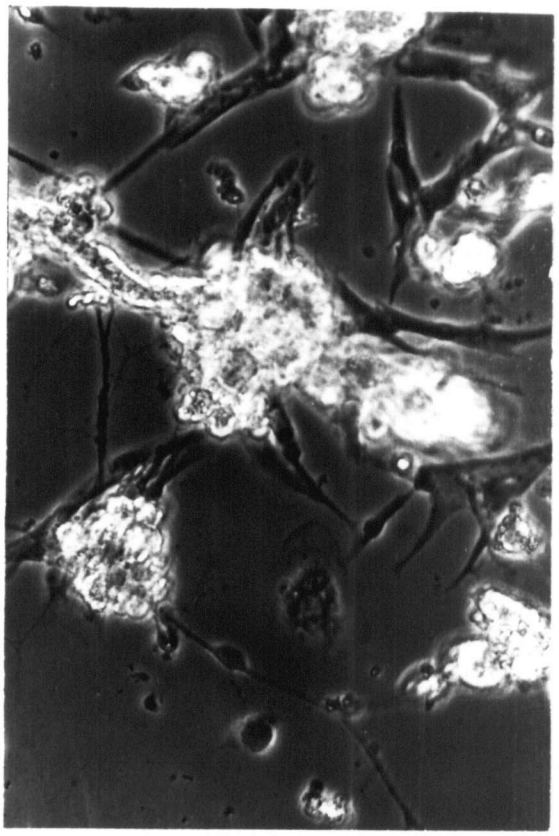
Figure 23. Percentage of, ○, ses E^1 mosaic survivors at 29°C; □, y w ses E^1/0 male survivors at 29°C and ●, y w ses E^+ mosaic survivors at 29°C.

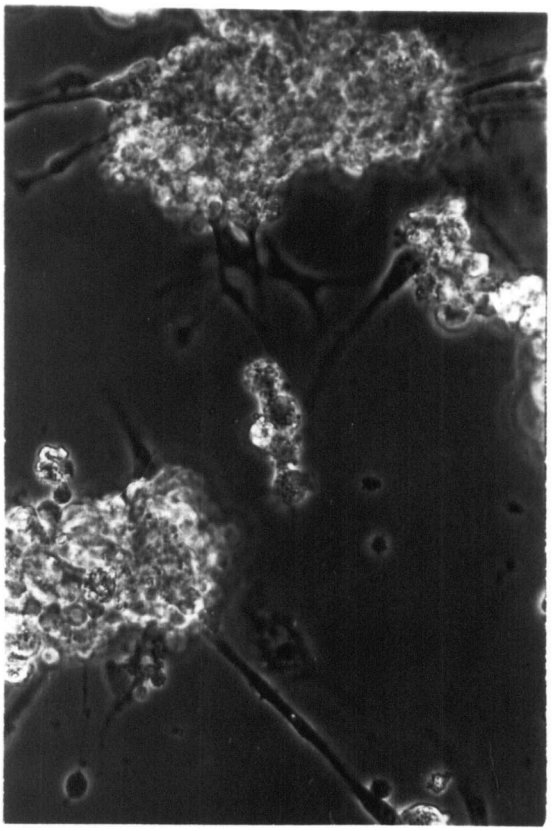

100
50
0
% adult survivors
5
10
15
20
days

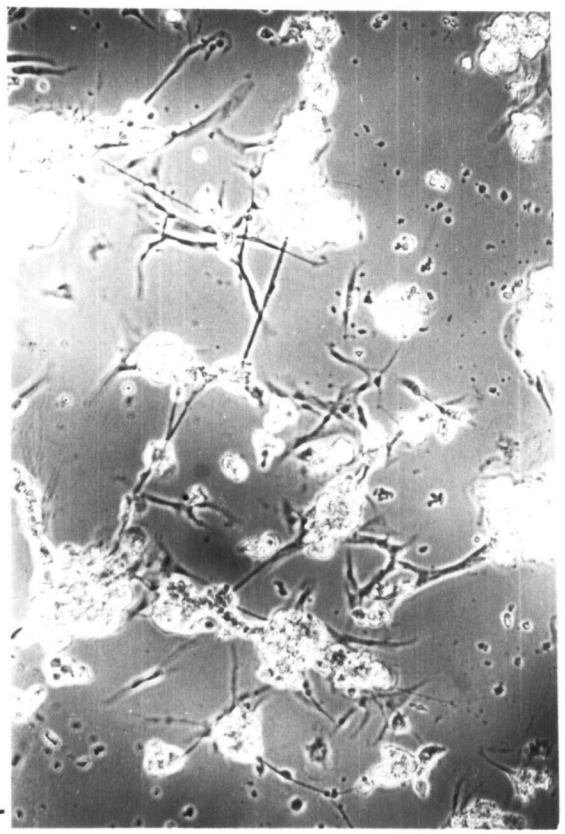
IV. Discussion

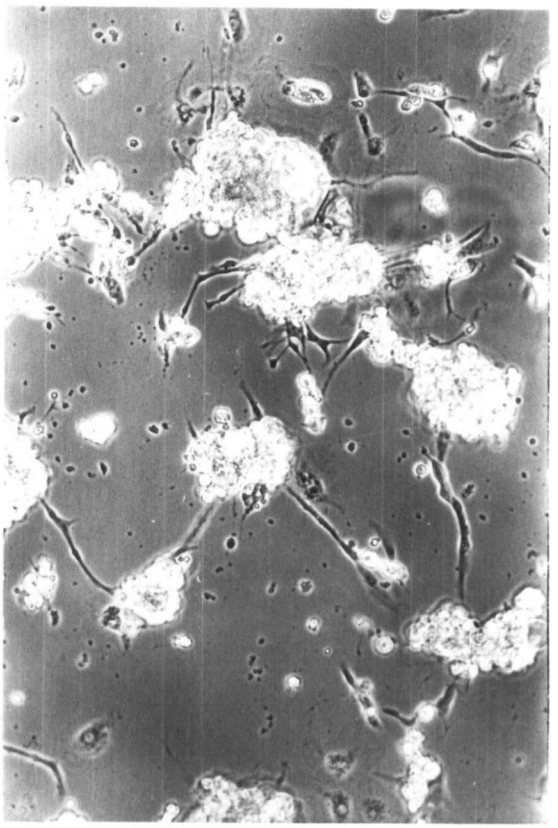
Temperature-shift studies indicate that the add A^{ts1} mutant has successive TSPs during the embryonic, 3rd instar larval-pupal and adult stages. The ses E^{1} mutant has TSPs between the 1st and 2nd larval instar and the 3rd instar larval-pupal stages and the adult stage. The embryonic and the 3rd larval-pupal stages represent periods of considerable activity of developmental genes (Hadorn, 1951) so these results are not surprising. The microscopic observations made on embryos of add A^{ts1} provide cytological verification of abnormal embryonic development. It was observed that the embryos develop normally until 10 hours after oviposition at which time differences between the Oregon-R and add A^{ts1} embryos were first visible with the light microscope. This coincides with the TSP inferred from shift studies. After 12 hours, an amorphous mass of tissue reminiscent of Notch embryonic lethals (Shellenberger and Mohler, 1978) appears. Earlier studies of add A^{ts1} embryonic tissue cultures revealed that there is a poor association of nerve and muscle cells, as well as a poor agglomeration of other different cell types (C. Beard, personal communication, Fig. 24). In addition to poor neuromuscular junction formation, the number of neural and muscle cells is reduced relative to the controls. These observations suggest that add A^{ts1} could affect both muscle and nerve development. Reduced numbers of nerve and muscle cells (Buzin et al., 1978) and poor development (Cross and Sang, 1978)

Figure 24. Cell cultures of I, Oregon-R at 29°C; II, same as I but at a higher magnification; III, add A^{tsl} at 29°C; IV, same as III but at a higher magnification.


II


IV


I


III

have also been observed with cell cultures of another embryonic lethal, shi^{ts1}. It is tempting, therefore, to suggest that the developmental abnormalities of add A^{ts1} stem from a defect in cell membranes that facilitate cell to cell connections. Among other possibilities, the lack of or reduced production of chemical signals in cells responsible for intercellular communication could result in some of the observed phenotypes. This in turn could be due to a lack of or a reduced level of enzymes which are responsible for synthesis, release or reception of such chemical signals. For example, Levi-Montalcini et al. (1968) demonstrated that a protein, called the nerve growth factor (NGF), plays an important role in the architecture of the nervous system both in vivo and in vitro.

Results from temperature-shift studies suggest the possibility of an altered protein. It is possible to test the existence of a diffusible ts gene product by mixing cell cultures from normal and mutant embryos and determining whether there is any influence of the wild type cells on the development of mutant myocytes and neurocytes. Ideally, a cell marker system would be incorporated into such an experiment so that the genotype of individual cells can be unequivocally determined. Unfortunately, such a system is not available at the present time. But, it is possible to compare relative cell counts before and after mixing with wild type cells (Cross and Sang, 1978).

Altogether, tissue culture and temperature-shift studies suggest a ts product which is essential for the development of nerve

and muscle cells most likely operates in add A^{tsl}. More concrete conclusions on the add A^{tsl} defect came from fate mapping analysis which indicate that its abnormalities lie in the muscular system. Therefore, the lack of coordination between the nerve and muscle cells in developing cell cultures could be attributed to a membrane defect because membranes are known to be important in cell to cell communications such as neuro-muscular junction formations during development.

If add A^{tsl} does affect skeletal muscles, then drugs affecting skeletal muscle neuro-transmissions would be expected to have an effect on add A^{tsl}. The fact that add A^{tsl} does not respond to any of these drugs other than drugs that affect the CNS and smooth muscles argues against the somatic muscle origin of this mutation. add A^{tsl} is more sensitive to Yohimbine, an adrenergic blocker, and is more resistant to pilocarpine nitrate, an agonist of cholinergic system. Both adrenergic and cholinergic system have an excitatory effect on the insect's CNS and smooth muscles (Pichon, 1974). Therefore, the existence of a relationship between add A^{tsl} drug responses and its muscular defect is doubtful. However, these results point to future pursuits in genetic mapping studies of the altered drug resistance phenotype. Unless mapping of this locus coincides with the drop-dead locus, the altered drug resistance could well be due to another locus affected by add A^{tsl}.

Mosaic analysis clearly indicated that the behaviour of each leg of add A^{tsl} mosaic was independent of the others. Also, once paralyzed, these legs often remained so during subsequent trials. These observations

suggest that the behaviour of each leg must has its own focus in the somatic muscular system responsible for controlling the movement of each leg.

Lethal mutations affecting muscle differentiation in Drosophila tissue cultures has been documented (Wright, 1960), but adult lethality due to muscular defect is still unheard of. In the present case, the seemingly unresolved results from drug studies and genetic mosaic analysis demand the need for confirmation of the muscular defect in add A^{tsl}. This can be done by using enzyme markers that will allow identification of mosaicism internally. Indeed, such techniques have been used to identify mosaicism in the nervous system of Drosophila (Kankel and Hall, 1976). These internal markers are usually null enzyme markers on the rod-X chromosome. Somatic loss of an unstable ring-X chromosome from the ring/rod heterozygote would lead to XO tissues which would not stain for the enzyme if these mosaics are scored internally by sectioning and histochemistry. It would be predicted that the absence of a certain type of mutant muscle cells would be found among survivors at 29°C. Unfortunately, an enzyme marker that will stain for muscles has yet to be discovered.

The other drop-dead mutation studied, ses E^1, is different from add A^{tsl} in that its defect is neural rather than mesodermal in nature. Fate mapping analysis indicates that the defect of ses E^1 mutation is in the nervous system. The demonstration of the neural basis for drop-dead mutations is not new. The drd lethality was

demonstrated both by histological and fate mapping studies to be due to a lesion in the brain tissues (Hotta and Benzer, 1972) while the dmd lethality was due to a defect in the thoracic ganglion (Flanagan, 1977). Drug studies showed that the ses E^1 mutant is more resistant to carbamylcholine chloride (an agonist of the cholinergic system) whose action in the insects' CNS is primarily excitatory on the post-synaptic cell (Pichon, 1974). This, together with temperature-shift studies, would suggest a cholinergic system playing a role in triggering certain signals that cause the release of, or synthesis of certain proteins which are essential both during and after development. The neural nature of this mutant directs future studies to fate map internal structures using the aforementioned enzyme marker technique in order to localize its defect more precisely.

Therefore, the add A^{ts1} gene product could be a component of the muscular system which fails to function at a higher temperature. The ses E^1 product seems to be involved in the synthesis of or the release of a thermolabile protein important in the functioning and integrity of the nervous system. To fully characterize these drop-dead mutations, more experiments are needed such as electron-microscopical studies on affected organs as suggested by fate mapping analysis, identification of a temperature-sensitive muscle protein biochemically using cultures of embryonic mutant cells (Storti et al., 1978) and binding studies of neurotransmitters to the isolated receptor sites (Eldefrawi, 1976).

LITERATURE CITED

Alder, J. (1975). Chemotaxis in bacteria. In: Ann. Rev. Biochem. ed. E. E. Snell, P. D. Boyer, A. Meister and C. C. Richardson. 44: 341-356.

Baillie, D., Suzuki, D. T. and Tarasoff, M. (1968). Temperature-sensitive mutations in Drosophila melanogaster, II. Frequency among second chromosome recessive lethals induced by ethyl methanesulfonate. Can. J. Genet. Cytol. 10: 412-420.

Baker, B. S. (1973). The maternal and zygotic control of development by cinnamon, a new mutant in Drosophila melanogaster. Devel. Biol. 33: 429-440.

Beament, J. W. L. (1958). A paralysing agent in the blood of cockroaches. J. Ins. Physiol. 2: 199-214.

Benzer, S. (1967). Behavioural mutants of Drosophila isolated by countercurrent distribution. Proc. Natl. Acad. Sci. (U.S.A.) 58: 1112-1119.

Bertler, A., Carlsson, A. and Rosengren, E. (1956). Release by reserpine of catecholamines from rabbits' hearts. Natwrwissenschaften 43: 521.

Bodenstein, D. (1950). The postembryonic development of Drosophila. In "Biology of Drosophila" ed. M. Demerec. p.175-367. John Wiley and Sons, New York.

Burn, J. H. (1960). Tyramine and other amines as noradrenaline-releasing substances. In "Adrenergic Mechanism, Ciba Foundation Symposium" p.326-336. Little, Brown and Co., Boston.

Burn, J. H. and Rand, M. J. (1957). Reserpine and noradrenaline in artery walls. Lancet 2: 1097.

Burn, J. H. and Rand, M. J. (1958). Noradrenaline in artery walls and its dispersal by reserpine. Brit. Med. J. 1:903-908.

Burn, J. H. and Rand, M. J. (1959a). Sympathetic postganglionic mechanism. Nature 184: 163-165.

Burn, J. H. and Rand, M. J. (1959b). The cause of the supersensitivity of smooth muscle to noradrenaline after sympathetic degeneration. J. Physiol. 147: 135-143.

Buzin, C. H., Dewhurst, S. A. and Seecof, R. L. (1978). Temperature-sensitivity of muscle and neuron differentiation in embryonic cell cultures from the Drosophila mutant, shibire^{ts1}. Develop. Biol. 66: 442-456.

Chan, L. -N. And Gehring, W. (1971). Determination of blastoderm cells in Drosophila melanogaster. Proc. Natl. Acad. Sci. (U.S.A.) 68: 2217-2221.

Colaianne, J. J. and Bell, A. E. (1972). The relative influence of sex of progeny on the lethal expression of the sonless gene in Drosophila melanogaster. Genetics 72: 293-296.

Cook, B. J. (1967). An investigation of Factor S, a neuromuscular excitatory substance from insects and crustacea. Biol. Bull. Woods Hole 133: 526-538.

Cook, B. J., de la Cuesta, M. and Pomonis, J. G. (1969). The distribution of Factor S in the cockroach, Periplaneta americana, and its role in stress paralysis. J. Ins. Physiol. 15: 963-975.

Cook, B. J. and Holt, G. H. (1974). Neurophysiological changes associated with paralysis arising from body stress in the cockroach, Periplaneta americana. I. Ins. Physiol. 20: 21-40.

Cross, D. P. and Sang, J. H. (1978). Cell culture of individual Drosophila embryos, II. Culture of X-linked embryonic lethals. J. Embryol. Exp. Morph. 45: 173-187.

Davey, K. G. (1963). The release by enforced activity of the cardiac accelerator of Periplaneta americana. J. Ins. Physiol. 9: 375-381.

Edgar, R. S. and Wood, W. B. (1966). Morphogenesis of bacteriophage T4 in extracts of mutant-infected cells. Proc. Natl. Acad. Sci. (U.S.A.) 55: 498-505.

Eldefrawi, A. T. (1976). The acetylcholine receptor and its interactions with insecticides. In "Insecticide Biochemistry and Physiology" ed. C. F. Wilkinson. Plenum Press.

Falk, D. R. and Nash, D. (1974). Sex-linked auxotrophic and putative auxotrophic mutant of Drosophila melanogaster. Genetics 76: 755-766.

Fausto-Sterling, A. (1971). On the timing and place of action during embryogenesis of the female-sterile mutants fused and rudimentary Drosophila melanogaster. Develop. Biol. 26: 452-463.

Feitelson, J. S. and Hall, L. M. (1975).. Genetic and Behavioural analysis of stress-sensitive mutants of Drosophila melanogaster. Genetics 80: s30.

Flanagan, J. R. (1977). A method for fate mapping the foci of lethal and behavioural mutants in Drosophila melanogaster. Genetics 85: 587-607.

Garcia-Bellido, A. and Merriam, J. R. (1969). Cell lineage of the imaginal discs in Drosophila gynandromorphs. J. Exp. Zool. 170: 61-76.

Garen, A. and Gehring, W. (1972). Repair of the lethal developmental defect in deep orange embryos of Drosophila by injection of normal egg cytoplasm. Proc. Natl. Acad. Sci. (U.S.A.) 69: 2982-2985.

Grigliatti, T. A., Hall, L., Rosenbluth, R. and Suzuki, D. T. (1973). Temperature-sensitive mutations in Drosophila melanogaster, XIV. A selection of immobile adults, Molec. Gen. Genet. 120: 107-114.

Grigliatti, T., Suzuki, D. T. and Williamson, R. (1972). Temperature-sensitive mutations in Drosophila melanogaster, X. Developmental analysis of the paralytic mutation, $para^{ts}$. Develop. Biol. 48: 1-24.

Hathaway, D. S. and Selman, G. G. (1961). Certain aspects of cell lineage and norphogenesis studied in embryos of Drosophila melanogaster with an ultra-violet micro-beam. J. Embryol. Exp. Morphol. 9(Part 2): 310-325.

Heisenberg, M. and Gotz, K. G. (1975). The use of mutations for the partial degeneration of vision in Drosophila melanogaster. J. Comp. Physiol. 98: 217=241.

Hadorn, E. (1951). Developmental action of lethal factors in Drosophila. Adv. Genet. 4: 53-85.

Helsop, J. P. and Ray, J. W. (1959). The reaction of the cockroach Periplaneta americana L. to bodily stress and DDT. J. Ins. Physiol. 3: 395-401.

Hilderbrand, J. G., Hall, L. M. and Osmond, B. C. (1979). Distribution of binding sites for ^{125}I-labeled -bungarotoxin in normal and deafferented antennal lobes of Manduca sexta. Proc. Natl. Acad. Sci. (U.S.A.) 76(1): 499-503.

Hinton, C. W. (1955). The behaviour of an unstable ring chromosome of Drosophila melanogaster. Genetics 40: 951-961.

Hochman, B. (1973). Analysis of a whole chromosome in Drosophila. C. S. H. Symp. Quant. Biol. 38: 581-590.

Homyk, Jr., T. and Sheppard, D. E. (1977). Behavioural mutants of Drosophila melanogaster, I. Isolation and mapping of mutations which decrease flight ability. Genetics 87: 95-104.

Homyk, T., Szidonya, J. and Suzuki, D. T. (1980). Behavioural mutants of Drosophila melanogaster, III. Isolation and mapping of mutations by direct visual observations of behavioural phenotypes. Molec. Gen. Genet. 177: 553-565.

Hotta, Y. and Benzer, S. (1970). Genetic dissection of Drosophila nervous system by means of mosaics. Proc. Natl. Acad. Sci. (U.S.A.) 67: 1156-1163.

Hotta, Y. and Benzer, S. (1972). Mapping of behaviour in Drosophila mosaics. Nature 240: 527-535.

Hotta, Y. and Benzer, S. (1976). Courtship in Drosophila mosaics: Sex-specific foci for sequential action patterns. Proc. Natl. Acad. Sci. (U.S.A.) 73: 4154-4158.

Howard, B.D., Merriam, J. R. and Meshul, C. (1975). Effects of neurotropic drugs on Drosophila melanogaster. J. Ins. Physiol. 21: 1397-1405.

Ikeda, K. and Kaplan, W. D. (1970a). Patterned neural activity of a mutant Drosophila melanogaster. Proc. Natl. Acad. Sci. (U.S.A.) 66: 765-772.

Ikeda, K. and Kaplan, W. D. (1970b). Unilaterally patterned neural activity of gynandromorphs, mosaic for a neurological mutant of Drosophila melanogaster. Proc. Natl. Acad. Sci. (U.S.A.) 67: 1480-1487.

Ikeda, K. and Kaplan, W. D. (1974). Neurophysiological genetics in Drosophila melanogaster. Am. Zool. 14: 1055-1066.

Jan, L. Y. and Jan, Y. N. (1976). L-glutamate as an excitatory transmitter at the Drosophila larval neuromuscular junction. J. Ins. Physiol. 262: 215-236.

Jan, Y. H. and Jan, L. H. (1978). Genetic dissection of short-term and long-term facilitation at the Drosophila neuromuscular junction. Proc. Natl. Acad. Sci. (U.S.A.) 75: 515-519.

Jockusch, H. (1966). Relations between temperature sensitivity, amino acid replacements, and quarternary structure of mutant proteins. Bioch. Biophys. Res. Commun. 24: 577-583.

Judd, B.H., Shen, M. W. and Kaufman, T. C. (1972). The anatomy and function of a segment of the X chromosome of Drosophila melanogaster. Genetics 71: 139-156.

Kankel, D. and Hall, J. C. (1976). Fate mapping of nervous system and other internal tissues in genetic mosaics of Drosophila melanogaster. Devel. Biol. 48: 1-24.

Kaplan, W. D. and Trout, W. E. (1969). The behaviour of four neurological mutants of Drosophila. Genetics 61: 399-409.

King, R. C. (1970). Ovarian development in Drosophila melanogaster. Academic Press. New York.

Levi-Montalcini, R. and Angeletti, P. U. (1968). Nerve Growth Factor. Physiol. Rev. 48(3): 534-569.

Levine, J. D. and Tracey, D. (1973). Structure and function of the giant motorneuron of Drosophila melanogaster. J. Comp. Physiol. 87: 213-235.

Lewis, E. B. (1978). A gene complex controlling segmentation in Drosophila. Nature 276(5688): 565-570.

McEwen, R. S. (1918). The reactions to light and gravity in Drosophila and its mutants. J. Exp. Zool. 25: 49-105.

Meezan, E. and Wood, W. B. (1971). Sequence of gene product interaction in bacteriophage T4 tail core assembly. J. Mol. Biol. 58(3): 685-692.

Mohler, J. D. (1977). Developmental genetics of the Drosophila egg, I. Identification of 59 sex-linked cistrons with maternal-effects on embryonic development. Genetics 85: 259-272.

Nomura, M. and Morgan, E. A. (1977). Genetics of bacterial ribosomes. Ann. Rev. of Genetics 11: 297-347.

Pak, W. L. and Grabowski, S. R. (1978). In "The Genetics and Biology of Drosophila" Vol. 2a, p.553-604.

Pak, W. L. and Pinto, L. H. (1976). Genetic approach to the study of the nervous system. Ann. Rev. Biophysics and Bioengineering 5: 397-448.

Parks, H. B. (1936). Cleavage patterns in Drosophila and mosaic formation. Ann. Entomol. Soc. Amer. 29: 350-392.

Pichon, Y. (1974). The pharmacology of the insect nervous system. In "The physiology of Insecta" Vol. IV. ed. M. Rockstein. Academic Press, New York and London.

Poulson, D. F. (1965). Histogenesis, organogenesis and differentiation in the embryo of Drosophila melanogaster. In "Biology of Drosophila" ed. M. Demerec. p.246. Hafner, New York.

Samaranayaka, M. (1974). Insecticide-induced release of hyperglycaemic and adipokinetic hormones of Schistocerca gregaria. Gen. Comp. Endoc. 24: 424-436.

Shellenbarger, D. L. and Mohler, J. D. (1978). Temperature-sensitive periods and autonomy of pleiotropic effects of $l(1)N^{ts1}$, a conditional Notch lethal in Drosophila. Develop. Biol. 62: 432-446.

Silbert, D. F. (1975). Genetic modification of membrane lipid. Ann. Rev. Bioch. 44: 315-339.

Silverman, M. and Simon, M. I. (1977). Bacterial flagella. Ann. Rev. Microbiol. 31: 397-419.

Sternburg, J. G. (1963). Autointoxication and some stress phenomena. Ann. Rev. Entomol. 8: 19-38.

Sternburg, J., Chang, S. C. and Kearns, C. W. (1959). The release of a neuroactive agent by the American cockroach after exposure to DDT or electrical stimulation. J. Econ. Ent. 52: 1070-1076.

Storti, R. V., Horovitch, S. J., Scott, M. P., Rich, A. and Pardue, M. L. (1978). Myogenesis in primary cell cultures from Drosophila melanogaster: Protein synthesis and actin heterogeneity during development. Cell 13: 589-598.

Suzuki, D. T. (1970). Temperature-sensitive mutations in Drosophila melanogaster. Science 170: 695-706.

Suzuki, D. T., Grigliatti, T. amd Williamson, R. (1971). Temperature-sensitive mutations in Drosophila melanogaster, VII. A mutation ($para^{ts}$) causing reversible adult paralysis. Proc. Natl. Acad. Sci. (U.S.A.) 68: 890-893.

Suzuki, D. T., Piternick, L. K., Hayashi, S., Tarasoff, M., Baillie, D. and Erasmus, U. (1967). Temperature-sensitive mutations in Drosophila melanogaster, I. Relative frequencies among gamma ray and chemically induced sex-linked recessive lethals and semi-lethals. Proc. Natl. Acad. Sci. (U.S.A.) 57: 907=912.

Suzuki, D. T. and Procunier, J. D. (1969). Temperature-sensitive mutations in Drosophila melanogaster, III. Dominant lethals and semi-lethals on chromosome 2. Proc. Natl. Acad. Sci. (U.S.A.) 62: 369-376.

Tarasoff, M. and Suzuki, D. T. (1970). Temperature-sensitive mutations in Drosophila melanogaster, VI. Temperature effects on development of sex-linked recessive lethals. Develop. Biol. 23(3): 492-509.

Wright, T. R. F. (1960). The phenogenetics of the embryonic mutant lethal myospheroid in Drosophila melanogaster. L. Exp. Zool. 143: 77-99.

Zalokar, M., Audit, C. and Erk, I. (1975). Developmental defects of female-sterile mutants of Drosophila melanogaster. Develop. Biol. 47: 419-432.

APPENDIX

% pupae formation from hatched eggs developed under various concentrations of drugs.

DRUGS	CONC.	STRAINS						
		Ore-R	ses B^1	ses B^2	ses D^1	ses D^2	ses E^1	add A^{ts1}
Control no drugs		47*	75	51	85	68	100	58
		50	84	95	95	80	57	51
		83	85	78	97	78	96	45
		87	-	-	56	-	92	88
		72	-	-	-	-	31	-
Carbamyl-choline chloride	.75	74	86	77	92	92	82	65
	1.5	67	68	89	-	100	69	44
	3.0	33	64	50	68	87	89	36
	6.0	0	16	-	47	29	61	0
	12.0	0	0	0	0	0	70	0
Pilo-carpine nitrate	.2	72	80	51	75	-	97	80
	.4	62	82	-	53	-	-	-
	.8	60	-	3	35	-	-	71
	1.6	0	21	0	29	-	-	56
	3.2	0	-	0	45	-	-	63
Succinyl-choline chloride	.625	32	60	90	100	-	63	25
	1.25	70	80	94	-	68	83	70
	2.5	49	92	85	100	3	65	67
	5.0	78	93	86	90	50	85	46
	10.0	22	80	17	67	36	25	24
Nicotine	.0156	84	88	81	97	81	81	63
	.03125	94	97	64	75	100	71	60
	.0625	71	74	0	58	67	69	68
	.125	3	34	0	13	0	9	8
	.25	0	6	0	0	0	0	3
Atropine sulfate	1.5	57	63	56	95	83	88	63
	3.0	31	30	74	87	77	61	56
	6.0	6	21	24	46	60	78	28
	12.0	0	4	0	-	18	10	34
	24.0	0	0	0	0	0	0	-
Hexa-methonium bromide	.5	84	-	57	-	-	90	-
	1.0	88	83	40	90	-	95	70
	2.0	21*	45	13	83	-	93	69
	4.0	0	15	7*	80	-	43	11
	8.0	0	0	0	0	-	0	0
d-Tubocurare	1.0	80	64	79	69	80	92	73
	2.0	34	79	75	81	84	88	74
	4.0	86	100	50	69	70	84	74
	8.0	50	33	0	52	14	41	0
	-	-	-	-	-	-	-	-

DRUGS	CONC.	STRAINS						
		Ore-R	ses B^1	ses B^2	ses D^1	ses D^2	ses E^1	add A^{tsl}
Eserine sulfate	.00625	0	83	85	76	72	82	100
	.0125	0	75	55	53	67	90	59
	.025	0	82	76	-	90	45	63
	.05	0	-	63	3	34	3	13
	.1	0	3	82	0	0	0	5*
Reserpine	.05	33	19	20	-	-	60	0
	.1	54	3	5	62	-	100	78
	.2	17	0	-	13	-	-	0
	.4	3	0	-	-	-	62	0
	.8	0	0	0	26	-	0	0
Nialamide	.25	91	87	79	100	-	80	54
	.5	21	92	92	88	90	97	61
	1.0	67	66	58	95	100	77	58
	2.0	-	79	-	51	-	28	71
	4.0	0	75	21	87	50	62	32
Iproniazid	.25	60	83	82	68	85	80	55*
	.5	40	68	47	64	33	63	68
	1.0	34*	21	40	38	29*	39	46
	2.0	0	27	23	23	53	26	54
	4.0	0	0	5	3	5*	11*	18
Caffeine	.125	33*	53*	68	66	-	87	33
	.25	60	13	0	20	-	88	48
	.5	73	51*	90	80	-	95	28
	1.0	18*	0	0	67	-	83	13
	2.0	79	0	0	-	-	2	5
Theo-phylline	.125	73	88	78	70	-	87	43
	.25	-	-	-	70*	-	-	-
	.5	50	75	46*	59*	-	-	55*
	1.0	13*	0	0	16	-	0	0
	2.0	0	0	0	0	-	61*	0
Strychnine sulfate	.5	62	83	71	88	-	68	100
	1.0	29*	83	92	98	-	80	28
	2.0	0	35	0	54	-	83	42
	4.0	0	27	0	-	-	38	36
	8.0	0	-	46	43	-	45	0
Picro-toxin	.0025	36	56	95	88	100	90	43
	.005	58	75	86	95	75	87	44
	.01	50	78	82	82	69	55	64
	.02	67	69	42	76	57	90	26
	.04	41	11	24	15	21	50	23

DRUGS	CONC.	STRAINS						
		Ore-R	ses B^1	ses B^2	ses D^1	ses D^2	ses E^1	add A^{ts1}
Allyl-	1.25	58*	71*	80*	25*	38*	64*	5*
glycine	2.5	72*	10*	49*	0	0	50*	0
	5.0	2*	0	16*	0	0	0	0
	10.0	0*	0	0	0	0	0	0
	-	-	-	-	-	-	-	-
Amino	.2	52	88	66	36	46	84	33
oxyacetic	.4	0	35	37	86	73	90	0
acid	.8	0	35	30	0	0	92	0
	1.6	11*	0	0	0	0	0	0
	3.2	-	0	0	0	0	0	0
Methionine	.125	42	89	56	85	59	53	35
sulfoximine	.25	54	58	36	30	37	58	5
	.5	66	81	73	75	80	-	42
	1.0	16*	58	81	28	47	46	5
	2.0	28	68	55	53*	7	73	0
Sodium	.125	76	87	65*	95	-	66	59
barbital	.25	66	78	41*	93	-	77	48
	.5	62	71*	-	63*	-	-	36*
	1.0	-	-	0	81*	-	72	0
	2.0	11	0	0	0	-	0	0
Urethane	.25	77	92	73	97	73	66	26
	.5	64	95	50	83	76	84	40
	1.0	61	92	53	57	95	73	38
	2.0	13	8	0	0	0	5	0
	4.0	0	0	0	0	0	0	0
Dopamine	1.25	57	72	85	92	90	82	43
	2.5	-	90	55	85	85	55	55
	5.0	77	66	76	90	79	-	44
	10.0	58	76	63	58	-	54	15
	20.0	8	7	82	0	31	0	0
dl-Nor-	1.75	53	72	60	87	74	83	45
epinephrine	3.5	42	65	63	85	100	86	34
	7.0	4	57	66	56	95	56	25
	14.0	23	21	46	30	51	42	8
	-	-	-	-	-	-	-	-
Tyramine	.75	-	90	90	-	-	-	63
	1.5	100	82	58	-	-	55*	54
	3.0	26	36	45	22	-	40	63
	6.0	0	0	3*	0	-	0	3
	12.0	0	0	0	0	-	0	0

STRAINS

DRUGS	CONC.	Ore-R	ses B^1	ses B^2	ses D^1	ses D^2	ses E^1	add A^{ts1}
dl-	2.5	64	77	92	97	73	87	54
Octopamine	5.0	61	44	78	95	100	89	0
	10.0	31	56	70	18*	76	39	10
	20.0	0	11	9	0	0	0	0
	-	-	-	-	-	-	-	-
Histamine	1.8	54	89	62	-	0	-	65
	3.7	-	84	-	-	90	87	83
	7.5	62	-	-	-	0	15	22
	15.0	11	38	38	0	52	5	0
	30.0	0	0	-	0	0	0	0
Gama	6.0	-	92	91	-	-	-	-
amino-	12.0	-	-	88	-	70	-	79
butyric	25.0	13	61	56	80	59	42	10
acid	50.0	-	-	0	-	-	-	-
	-	-	-	-	-	-	-	-
Glutamic	6.25	55	81	66	100	87	97	33
acid	12.5	61	47	69	-	72	85	63
	25.0	37	56	61	80	92	96	60
	50.0	37	33	22	33	28	8	27
	100.0	11	0	0	13	0	0	0
Catechol	1.0	90	78	78	49	73	80	38
	2.0	82	92	49	72	95	81	18
	4.0	31	0	0	21	20	30*	0
	8.0	0	0	0	0	0	0	0
	16.0	0	0	0	0	0	0	0
Yohimbine	.5	89	66	39	61	93	88	16*
	1.0	54	72	0	42	86	53	11
	2.0	51	45	16	26	62	24	0
	4.0	0	0	0	0	0	0	0
	8.0	0	0	0	0	0	0	0

All experiments have over 80% adult/pupae with the exception of *, which have less than 50% adult/pupae. -, data not available due to either extensive mould growth in culture or experiments not performed.
All experiments were performed at 22°C.
All mutant strains fail to develop at 29°C.