THE TIME OF ACTION OF THE GENE *ANTENNALESS* AND ITS EFFECT ON THE DEVELOPMENT OF THE CEPHALIC COMPLEX OF *DROSOPHILA MELANOGASTER*

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(With Four Text-figures)

1. INTRODUCTION

The mutant gene *antennaless* first appeared in a rough-eyed stock of *Drosophila melanogaster* belonging to Dr Cecil Gordon. Under suitable environmental conditions, it gives rise to flies with no antennae (A_0). In other circumstances, flies of the same pure line may have two normal antennae (A_2) or one antenna (A_1). Intermediate types are rare. Some of the external conditions which influence exhibition of the gene have been examined by Gordon & Sang (1941), who have shown that it is possible to alter the proportion of the three phenotypes formed in a single culture by rearing the larvae at different temperatures. This has enabled them to determine the *temperature effective period* (T.E.P.) of the gene. The critical phase, found to occur about 3 days after hatching in this instance, is commonly taken to be identical with the time of action of the gene (Goldschmidt, 1938). The present investigation is an attempt (*a*) to find if the time of action so determined can be confirmed by other methods, and (*b*) to examine the general effects on a single organ produced by a single gene particularly sensitive to environmental conditions.

We have employed morphological examination, measurement of the organ affected, and the use of new culture media for the determination of the time of action of the gene. A comparison of the development and of growth rates of the frontal sacs in the different phenotypes of genotypically identical larvae gives a measure of the nature and time of divergence of *antennaless* from the normal. Such observations can be made entirely on A_1 larvae. Further, by successive implantation of larvae into media known to encourage high and low levels of gene exhibition, we can find the time at which the developing organ becomes autonomous with respect to the environment in question.

2. MATERIALS AND METHODS

(*a*) *Egg collection and sterilization technique.* The method of egg collection and sterilization is identical with that used by Gordon & Sang (1941) and Robertson & Sang (1944), except that we used twice the usual amount of $HgCl_2$, since the ordinary prescription (White's fluid) was inadequate to ensure sterility in all cultures. Larvae aged less than 8 hr. grew in sterile media described below.

(*b*) *Sterile media.* The three media used in the experiments to be described had the following compositions:

*M_1	Dried brewers' yeast	$\frac{1}{3}$ g.
	Sieved sawdust...	$\frac{2}{3}$ g.
	Pearl S 101 salt solution + 3 % dextrose	4 ml.
*M_2	'Perolin' bakers' yeast...	$\frac{1}{3}$ g.
	Sieved sawdust...	$\frac{2}{3}$ g.
	Pearl S 101 salt solution + 3 % dextrose	4 ml.
M_3	Dried brewers' yeast	$\frac{1}{3}$ g.
	Sieved sawdust...	$\frac{2}{3}$ g.
	Pearl S 101 salt solution + 24 % dextrose	4 ml.

* See Gordon & Sang (1941).

The vials containing the medium were autoclaved for 20 min. at 20 lb. pressure, the larvae being then transferred to them by means of sterilized platinum spoons.

(*c*) *Indices of gene exhibition.* Gordon & Sang (1941) made use of three indices of exhibition (α, β, γ) to express the proportions of the various phenotypes found in a given culture. If A_0, A_1, A_2 stand for the number of homozygous flies of *antennaless* stock having no antennae, one antenna or two antennae respectively, then

$$\alpha = \frac{A_0 + A_1}{A_0 + A_1 + A_2}, \quad \beta = \tfrac{1}{2}(\alpha + \gamma), \quad \gamma = \frac{A_0}{A_1 + A_2 + A_0}.$$

These same indices will again be used as a measure of the characteristics of a culture.

(*d*) *Histological technique.* Larvae removed from the standard sterile medium (M_1) by flotation in a sterile salt solution of high specific gravity, were fixed in Kahle's fluid for 1 hr. at 60° C. Dehydration prior to embedding in wax was then carried out according to the following routine already described in D.I.S. (1941).

1. 70 % alcohol	Overnight
2. 30 % alcohol	1 hr.
3. 45 ml. 45 % alcohol + 5 ml. normal butyl alcohol ...	2 hr.
4. 42 ml. 62 % alcohol + 8 ml. normal butyl alcohol ...	2 hr.
5. 32 ml. 77 % alcohol + 17 ml. normal butyl alcohol ...	4 hr.

6.	22 ml. 90% alcohol + 27 ml. normal butyl alcohol ...	Overnight
7.	12 ml. 90% alcohol + 37 ml. normal butyl alcohol ...	4 hr.
8.	Normal butyl alcohol ...	Overnight
9.	Normal butyl alcohol ...	Overnight or till required

Sections were stained in Mallory's phosphotungstic acid haematoxylin.

(*e*) *Measurement technique.* In order to measure the growth of the antenna buds, larvae of the required age were cleaned in a saline solution and dissected under a binocular. The head was pulled away from the body, and the frontal sacs (eye bud + antenna bud) separated off. After further dissection such preparations were fixed and stained in aceto-carmine and mounted in Farrant's medium. When a series of

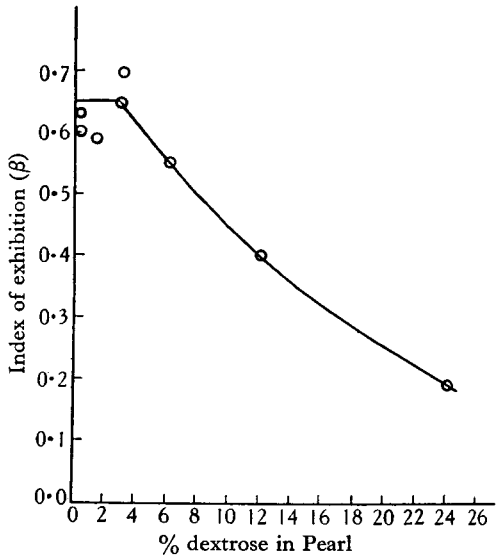

Fig. 1. Effect of dextrose on the exhibition of the gene *antennaless*.

preparations had been collected they were each projected, at constant magnification, on to a ground glass screen and photographed on to Kodak R.P. 30 oscillograph paper. The image of the antenna bud so obtained was cut out and the weight of the paper determined with a torsion microbalance. By reference to a photograph of 100 $\frac{1}{400}$ sq. mm. squares of a haematocrit similarly photographed the area represented by 1 mg. of paper was determined.

(*f*) *Environmental control of gene exhibition.* In the course of experiments on the effect of environment on gene exhibition, we found that high concentrations of dextrose greatly lowered gene exhibition (Fig. 1). This suggested that we could obtain a measure of the time of action of the gene by transferring larvae from a medium of low to one of high dextrose content. Accordingly, larvae of definite age were removed under sterile conditions from the low-sugar con[illegible] medium (M_1) on to sterile agar slabs. Traces of [illegible] salt flotation solution were removed from the larvae, as they crawled over these slabs. The larvae were then seeded on to a medium of high dextrose content (M_3). Controls were removed from the incubator while transfers were made. The sterility of all vials was tested at the time of transfer and at the end of the experiment. No infection occurred.

3. DEVELOPMENT OF THE CEPHALIC COMPLEX IN A_1 FLIES

Robertson (1936) and Chen (1929) have described the normal development of the frontal sacs of *D. melanogaster*. Here we shall only describe the relevant details of the development of a half-antennaless individual. For this purpose we assume that larvae showing lateral asymmetry of the developing buds would have given rise to half-antennaless imagos.

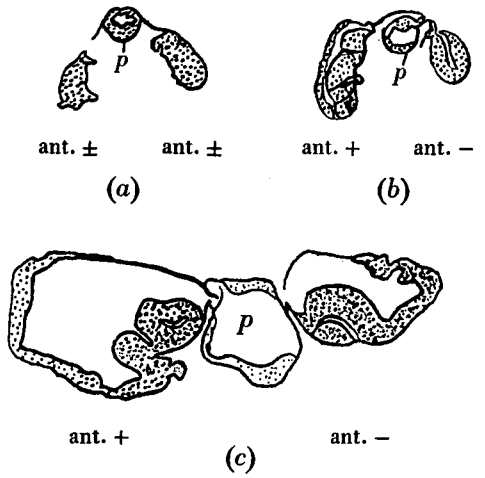

Fig. 2. Stages in development of the antenna buds of a half-antennaless larva: (*a*) about 70 hr., (*b*) 96 hr., (*c*) pupa just prior to fusion of frontal sacs. *ant.* + = normal antenna bud. *ant.* − = antennaless bud. *p* = pharynx.

The left and right antennal buds are not different in appearance up to about 70 hr. from the time of larval hatching. At this stage, both appear in cross-section as pear-shaped thickenings of the frontal sacs hanging down on either side of the pharynx (Fig. 2 *a*). The eye bud is not yet clearly marked off from the antenna bud and no folding or segmentation has yet taken place. The sacs are connected down their length by a thin sheet of tissue.

The effective developmental changes are visible after this and continue during the next 30 hr. Fig. 2 *b* shows the buds of a 96 hr. A_1 larva. There are now two differences between the wild type (*ant.* +) and antennaless (*ant.* −) sides. First, the normal bud (*ant.* +) is much larger than its fellow, the antennaless bud (*ant.* −). Models prepared at this stage show that

[redacted]ize difference mainly affects the diameter and not the length of the bud. This is what would be expected in view of how the antenna is formed (Chen, 1929). Second, the antennaless bud is unsegmented, while the wild type bud is segmented. Failure to segment in antennaless (*ant.*−) results in the absence of the 'nipple' found in the normal buds. Hence, the gene *antennaless* acts both on the normal growth of the bud, and on its differentiation.

At about 100 hr., growth of the antennaless (*ant.*−) bud appears to cease. Shortly before pupation, the anterior wall of the normal sac is found to be much thicker than that of the antennaless one. This segmented thickening is the antenna bud proper. Occasionally, the eye bud on the antennaless side also shows some diminution. This tallies with the observation that reduction of the imaginal eye sometimes occurs on the antennaless side.

As regards the cephalic complex, the prepupal phase is marked by medial fusion of the frontal sacs. This is true of all three phenotypes, and the process is completed about 12 hr. after pupation. Fig. 2 *c* shows a cross-section of an A_1 pupa just prior to this fusion. The antenna bud now consists of a segmented thickening about half way down the frontal sac. The antennaless sac is only slightly thickened. Eventually the frontal sacs are everted to form the head of the adult.

4. GROWTH RATE OF THE NORMAL AND ANTENNALESS CEPHALIC COMPLEX

Fig. 3 and Table 1 show the mean growth rates of *ant.*+ and *ant.*− buds. The normal growth curve was determined on two media (M_1 and M_2); the growth rate of the antennaless *anlage* was determined on M_1 only.

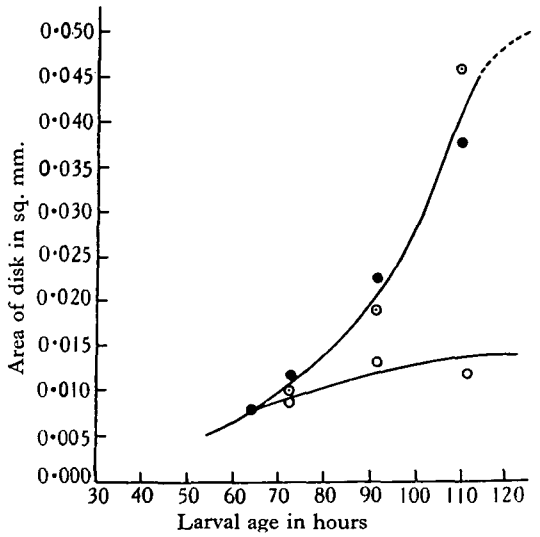

Fig. 3. Growth curves for normal (*ant.*+) and antennaless (*ant.*−) antenna buds. −●− = normal on M_1. −⊙− = normal on M_2. −○− = antennaless (*ant.*−) on M_1.

The graphs show that up to about 65 hr. the growth rate of normal (*ant.*+) and antennaless (*ant.*−) imaginal disks is approximately the same. Thereafter, the normal disk grows and differentiates rapidly following an exponential curve, while the antennaless disk grows very slowly. That is, the general conclusions reached by histological examination are confirmed. The time of separation of the growth curves gives an independent measure of the time of action

Table 1

Larval age hr.	Growth medium	Area of antenna disk (sq. mm.)	
		A_2	A_0
64¼	M_1	0·0076	0·0076
73¼	M_1	0·0115	0·0088
91¾	M_1	0·0223	0·0130
111½	M_1	0·0373	0·0118
72¾	M_2	0·0095	—
91¾	M_2	0·0189	—
111¾	M_2	0·0455	—

of the *antennaless* gene. Before 65 hr. we have no indications that the growth of the frontal sacs is in any way affected by the presence of this gene, but provided specific external influences, to be discussed later, do not intervene, growth slows down rapidly. In the presence of such substances, as in the normal wild type, growth continues exponentially.

5. ENVIRONMENTAL CONTROL OF GENE EXHIBITION

Fig. 4 and Table 2 show the results of transferring larvae of various ages from a sterile medium of low dextrose content (M_1) to a sterile medium of high

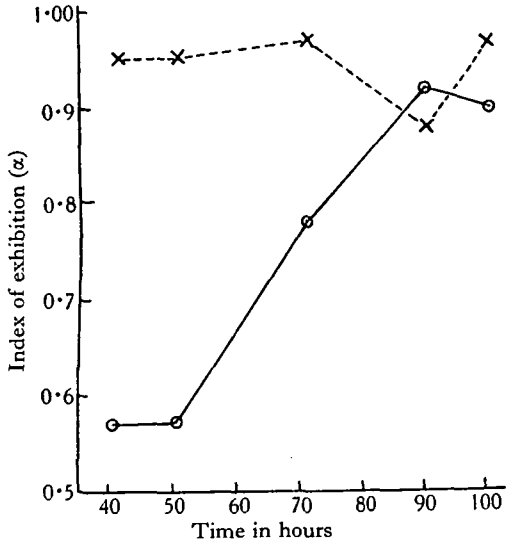

Fig. 4. Effective period of the gene *antennaless* by the dextrose technique. -- × -- = control cultures. −⊖− = experimental cultures.

dextrose content (M_3). The upper curve (--×--) shows indices for flies of the control group, grown throughout on M_1. The lower curve (—⊙—) shows indices for larvae transferred at 42, 51, 72, 90 and 100 hr. from M_1 to M_3. These two curves show that exhibition can be modified by alterations of the environment at all times prior to 60 hr. Between 60 and 70 hr., the proportion of animals which can be

Table 2

Age at transfer hr.	Index of exhibition					
	Control			Experimental		
	α	β	γ	α	β	γ
42	0·95	0·69	0·42	0·57	0·32	0·07
51	0·95	0·65	0·34	0·57	0·32	0·06
72	0·97	0·87	0·77	0·78	0·53	0·27
90	0·88	0·63	0·37	0·92	0·76	0·60
100	0·97	0·71	0·44	0·90	0·72	0·54

so affected declines rapidly. That is, most 75 hr. old larvae, at 25° C. on the particular medium (M_1) used, have reached a stage at which antenna determination is complete. Thereafter, antenna formation is no longer influenced by nutritional factors. On the basis of these experiments we may say that the effect of the gene can no longer be modified about 80 hr. after hatching.

DISCUSSION AND CONCLUSIONS

Many workers have studied the role of the gene in determining developmental processes (see Waddington, Beadle, Goldschmidt, etc.). In a number of instances the T.E.P., i.e. period during which the gene action may be altered by temperature changes, has been determined and this has been identified with the time of action of the gene. Recently, Child (1940) has pointed out some difficulties in interpreting results from experiments of this kind.

We have been able to determine two limits:

(*a*) The earliest time at which the *antennaless* gene manifestly affects the course of antennal development, as shown by measurement and gross histological examination.

(*b*) The latest time at which it is still possible to modify development by means of specific nutritional substances.

The effects of the gene can be first discerned about 70 ± 5 hr. after the larva hatches and this agrees well with the T.E.P. found by Gordon & Sang (1941). It is impossible to modify the development after about 80 hr. Hence, our two limits (*a*) and (*b*) lie very close together and are probably not more than 12 hr. apart for an individual larva.

On the assumption that this critical phase represents the time of action of the gene, it would be reasonable to say that this also corresponds to the T.E.P. However, the first assumption is an arbitrary one, since we have no means of knowing how early in development the gene actually becomes physiologically active. This initial action need not necessarily result in specific physiological changes in the developing organ (e.g. the tissue may not yet be 'competent') and for this reason it may be better to talk of the 'effective period' instead of the 'time of action of the gene'.

SUMMARY

A. The development of the antenna buds of the mutant *antennaless* of *D. melanogaster* has been examined and it is shown that:

(1) Prior to about 70 hr., no histological difference is detectable as between normal and abnormal *anlagen.*

(2) Normal antenna buds continue to grow exponentially after 70 hr.; abnormal buds scarcely grow at all.

(3) The abnormal bud is also distinguished from wild type by its failure to segment.

(4) Both normal (*ant.* +) and antennaless (*ant.* −) frontal sacs are everted normally in the pupa.

B. The period during which the course of development of antennaless buds of the same genotype could be modified by specific nutritional substances was also determined. It is shown that media containing a high concentration of dextrose will induce a lowered exhibition. By transferring larvae from normal media to media of high dextrose content, it is found that the course of development of the antenna bud cannot be modified after about the 80th hr. of larval life.

These experiments suggest that the *effective period* of the gene is relatively short and is probably not more than 12 hr. for each individual larva. This period corresponds to the T.E.P., but is not necessarily identical with 'the time of action of the gene'.

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