clearly reveals the corpora allata and ovary interaction. But the question why the level of the 2 enzymes showed a maximum decrease after both corpora allata and brain operation remains unanswered.

The role of juvenile hormone on the GOT and GPT activity has been proved by injecting the juvenile hormone analogue into allatectomized insects, which reverses the effect of allatectomy. But the results after the application of juvenoid to allatectomized insects for the different tissues in both sexes are different, and this signifies that the sensitivity of the different tissues and sexes to the juvenile hormone analogue used here were different. The results obtained here show that the tissues most sensitive to juvenile hormone analogue action and those with the greatest potential for response, are the fat body and the ovary, and to some extent the hemolymph, too. It was also shown that the females were more sensitive to the juvenoid than the males. The main cause for this type of differential response to juvenoid and other hormone action will be clear when the molecular mode of hormone action on insects is discovered.

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Evidence for the neurohormonal basis of commitment to pupal diapause in larvae of *Sarcophaga argyrostoma*[1]

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Summary. Implantation of brain-ring gland complexes from short-night larvae into long-night larvae reversed the diapause 'programme' of the recipients after metamorphosis to the pupal stage. The converse experiment did not induce diapause. These results demonstrate that the larval endocrine centers are photoperiodically programmed for diapause or non-diapause development long in advance of the diapause stage.

One of the more important unresolved problems in insect developmental biology is how environmental factors (specifically photoperiod) induce seasonally appropriate commitments to diapause or non-diapause development. In the flesh fly, *Sarcophaga argyrostoma,* for example, larvae raised in short daylengths (long nights) enter an overwintering pupal diapause, whereas those raised in long days (short nights) adopt an alternative summer pathway with uninterrupted pupal development and successive generations of flies[2-4].

The minimal requirements for such a response are a photoreceptor, a 'clock' to measure daylength (or nightlength) and to integrate such information, and an effector system to control the onset of the diapause state. Investigations into the nature of the photoperiodic clock are still restricted to largely formal analyses: these show that nightlength is the component of the environment that is measured, and that the clock is part of the insect's circadian system[5-8]. Next to nothing is known about the concrete physiology of time measurement, and this situation is unlikely to change until more is known about the physiology of circadian pacemakers. On the other hand, there is some concrete knowledge of the hormonal basis of the diapause state. Several investigators have shown that pupal diapause in *Sarcophaga* spp. may be terminated by an administration of exogenous ecdysteroids[9-11], and it is thought that pupal diapause in these flies is a result of the inactivation of the cerebral neurosecretory cells, a consequent hiatus in the secretion of the prothoracotropic hormone (PTTH), and a halt to ecdysone production by the ring gland[12]. Diapause occurs at a precise morphogenetic stage[9] in a 'trough' between 2 periods of ecdysteroid release, the former correlated with puparium formation and pupation, the second initiating adult differentiation[13]. In diapause pupae, therefore, the second, adult-initiating pulse of ecdysterone does not occur and development ceases[14].

The most obvious gap in our understanding is how the clock, having discriminated between a short and a long night, then controls the secretion or retention of PTTH.

This problem is compounded by the fact that the photoperiodic clock is functional during embryonic and early larval development[4,15], but the diapause occurs in the pupa. The mechanism thus involves the measurement and accumulation of inductive light cycles during the 'sensitive period'[3] and a transfer of this information through a period of intense metamorphic activity.

For this reason we have directed our attention to the larvae, specifically to stages near the end of the sensitive period, but before puparium formation. Using such post-fed larvae, Denlinger[16] has already shown that pupal diapause may be prevented by the administration of ecdysterone. We now ask whether the diapause-committment of larvae at the end of the sensitive period can be changed by implantation of neuroendocrine tissues from donors programmed for the alternative developmental pathway.

Materials and methods. Adults of *S. argyrostoma* were kept at 25 °C, in either long nights (LD 12:12) or in continuous light (LL). Larvae produced by long-night flies were then raised in LD 12:12, 19-20 °C, on a diet of meat supplemented by a dried milk-yeast-agar medium to provide a stock of diapause-programmed larvae. Those produced by LL females were raised in short nights (LD 18:6, 19-20 °C) to provide larvae committed to uninterrupted development. 3rd instar 'wandering' larvae, 3 days after leaving their food, were used in these experiments.

The neuroendocrine system of a *Sarcophaga* larva is shown in figure 1. As in all higher flies (Diptera, Cyclorrhapha) the central nervous system consists of a brain and a ventral nerve mass condensed to a trilobed structure perforated by the oesophagus. Lying above the brain and surrounding the aorta is the ring gland which comprises the 3 major components of the insect endocrine system: the corpus cardiacum, the lateral prothoracic glands, and the corpus allatum.

Entire brains plus ring glands (CNS + RG), brains with the prothoracic gland and corpus allatum cut away (i.e. brains plus corpus cardiacum, CNS + CC), brains without ring glands (CNS), and ring glands alone (RG) were removed from donor short-night (development-committed) or long-night (diapause-committed) larvae and placed in cold (4 °C) insect Ringer before implantation into the abdomens of long-night (diapause-committed) recipients anesthetized on ice for about 1 h. Larvae received 1, 2 or 3 such implants. In another series, 3 entire brain-ring gland complexes (CNS + RG) from donor long-night (diapause-committed) larvae were implanted into short-night (development-committed) recipients. Sham operated controls were injured in the same way as the experimental larvae but received no implants. Other control groups were simply immobilized on ice. Experimental and control larvae were then returned to their previous conditions of photoperiod and temperature and allowed to form puparia in the normal way. After 10-14 days the puparia were opened to ascertain whether they contained undeveloped diapause pupae or pigmented pharate adults.

Results and discussion. Implantation of 3 CNS + RG from diapause-destined (long-night) larvae into development-committed (short-night) recipients failed to induce diapause. All 19 such larvae developed to the adult stage. This result excluded the possibility that a specific 'diapause

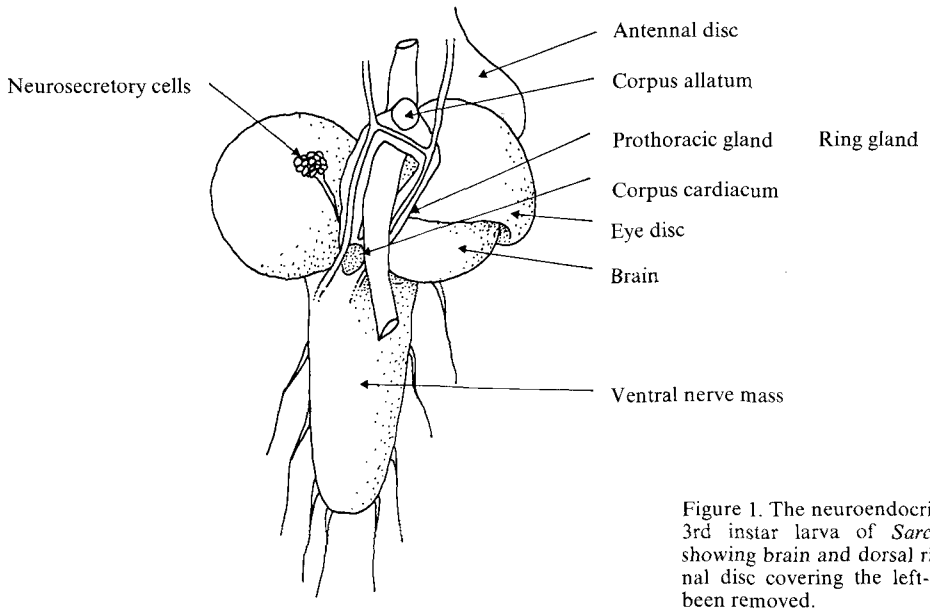

Figure 1. The neuroendocrine system of an early 3rd instar larva of *Sarcophaga argyrostoma*, showing brain and dorsal ring gland. The imaginal disc covering the left-hand brain lobe has been removed.

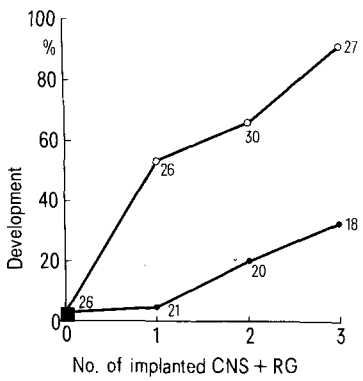

Figure 2. The development-inducing effects of implantation of entire brains plus ring glands (CNS + RG) into diapause-programmed larvae of *Sarcophaga argyrostoma*. ○, donors programmed for non-diapause development by short nights. ●, donors programmed for pupal diapause by long nights. ■, sham-operated control (no implants). Numbers close to plotted points are the sample sizes.

The development-inducing effects of implantation of various portions of the neuroendocrine system from development- to diapause-programmed larvae of *Sarcophaga argyrostoma*

Implant	Number of larvae	Pupae developing to adult flies	
		Number	%
3 CNS+RG	27	25	92.6***
3 CNS+CC	13	5	38.5**
3 CNS	22	1	4.5 n.s.
3 RG	22	2	9.1 n.s.
Sham operated	26	1	3.8
Cold narcosis	26	1	3.8

CNS+RG, brain plus entire ring gland; CNS+CC, brain plus corpus cardiacum (i.e. with prothoracic gland and corpus allatum cut away); CNS, brain without ring gland; RG, ring gland alone. ***$p < 0.001$; **$p < 0.01$; n.s., not significant, compared with sham operated control.

hormone' is involved in programming diapause in *Sarcophaga*. On the other hand, implantation of 1, 2 or 3 CNS+RG from development-destined (short-night) larvae into diapause-committed (long-night) recipients, stimulated their development in a 'dose-dependent' fashion (fig. 2). Control implants from donors previously programmed for diapause by a series of long nights stimulated development to a much lesser degree. For example, in each pair of observations (1, 2 or 3 complexes) development was significantly higher for larvae receiving development-committed (short-night) implants (1 implant, $\chi^2 = 12.87$, $p < 0.001$; 2 implants, $\chi^2 = 10.47$, $p < 0.01$; 3 implants, $\chi^2 = 17.70$, $p < 0.001$), and 1 development-committed complex altered the diapause programme more strongly than 3 diapause-committed ones.

Our results thus show that clear differences exist between the complexes of larvae programmed for continuous development or for diapause, indicating that the endocrine potentials of such complexes are differentiated several days before diapause occurs. In this respect these observations differ from those in which endocrine differences immediately responsible for diapause or development were demonstrated[17].

To find out which portions of the neuroendocrine complex contribute to the stimulation of development, we implanted 3 CNS with corpora cardiaca (CNS+CC) from short-night donors into long-night recipients. This treatment prevented diapause in only 5 (38.5%) of the 13 insects (table). Furthermore, brains alone (CNS) or ring glands alone (RG) had no significant effect. Thus an intact PTTH-ecdysone axis is important for preventing diapause, which might suggest that prothoracotropic activity of the brain and the production of ecdysteroids were enhanced in the larvae committed for development by short nights in comparison with those committed for pupal diapause by long nights.

Our present results suggest that implanted complexes might change the hormonal balance in the recipients, in turn effecting feedback interactions[18] between the levels and timings of PTTH, ecdysteroids (and perhaps juvenile hormones)[12,19] which together regulate diapause or development in the pupa. On the other hand, implanted complexes might merely survive metamorphosis and produce their hormones, as programmed, to initiate adult development in otherwise diapausing pupae.

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Allophenic mice produced from embryos aggregated with antibody[1]

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Summary. One difficulty in the production of allophenic mice by aggregation of preimplantation embryos is that they frequently roll apart before the bonds between the blastomeres have had time to form. One solution to the problem, described here, is to pretreat one of the embryos with rabbit anti-mouse serum just prior to pushing them together. Blastocyst formation is unhampered by antibody treatment, and numerous allophenic mice have already been produced with this new procedure.

Mice composed of genotypically distinct populations of cells frequently reveal information unobtainable from studies of their pure strain counterparts. Allophenic mice represent one class of such genetic mosaics, and they are routinely produced in vitro by aggregation of preimplantation embryos from 2 strains of mice[3]. These composite embryos are then allowed to develop to term in the uteri of pseudopregnant foster mothers and thereby become fully developed mice, all of whose tissues usually contain cells from each strain[4]. The Ist part of the procedure involves