

## INHIBITION OF OÖCYTE DEVELOPMENT DURING PREGNANCY IN THE COCKROACH *EUBLABERUS POSTICUS*

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(Received 17 July 1972)

**Abstract**—The corpora allata are inhibited during pregnancy in ovoviviparous *Eublaberus posticus*, and yolk is not deposited in the basal oöcytes for the entire or almost the entire gestation period.

Precocious oöcyte development occurs if the oötheca is removed but this can be prevented by substituting a plastic oötheca for the true egg case in the uterus. Implantation of a uterus containing an oötheca into the abdomen of a female whose oötheca is removed does not prevent precocious oöcyte development even though many of the eggs in the implant grow and stretch the donor uterus. These experiments argue against the hypothesis that an 'agent' from the uterine eggs or stretched uterus inhibits the activity of the corpora allata (CA), and supports the hypothesis that inhibition from the uterus is mechanical.

Cyclical activity of neurosecretory cells in certain abdominal ganglia in one species of ovoviviparous cockroach has been correlated with the cyclical inhibition of the oöcytes during pregnancy. Mechanoreceptors are found in the uteri of several ovoviviparous species including *Eublaberus*.

In *Eublaberus* transecting the nerve cord between various ganglia in pregnant females only results in a marked decrease in the percentage of females showing precocious oöcyte development when the nerves posterior to the sixth abdominal ganglion are severed. However, the results are the same if these nerves are severed after removing the oötheca. It is suggested that pressure of the oötheca on mechanoreceptors in the uterus, or cessation of pressure (after removal of the oötheca), result in sensory information being transmitted to the last abdominal ganglion which affect the CA, perhaps indirectly by controlling the activity of the neurosecretory cells in various abdominal ganglia.

### INTRODUCTION

DURING pregnancy in ovoviviparous cockroaches, the corpora allata (CA) are inhibited for the entire or almost the entire gestation period (Roth, 1970). As a result, the basal oöcytes of the ovary do not accumulate yolk. The first attempt to explain this inhibition was made by LÜSCHER and ENGELMANN (1955) and ENGELMANN (1957) who suggested that developing eggs (probably the yolk system) in the brood sac of *Leucophaea maderae* (F.) release a substance which causes the brain to inhibit the CA. However, ROTH and STAY (1959) hypothesized that inhibition of the CA during pregnancy was due to nervous stimuli resulting from pressure of the oötheca on the uterus in ovoviviparous *Pycnoscelus surinamensis* (L.) and on the vestibulum in *Blattella germanica* (L.) (an oviparous species which

carries its oötheca externally until the eggs hatch). The mechanical inhibition hypothesis was extended to include studies of several other species of cockroaches that incubate their eggs internally (ROTH and STAY, 1961, 1962a, b; ROTH, 1964a b).

Since the original hypothesis that uterine eggs probably produce an inhibitory agent during pregnancy, ENGELMANN (1960) has suggested (1) that the inhibitory action of the uterine eggs may be either humoral, or mechanical, or both; (2) that a specific or non-specific agent is released by the egg case or brood sac and acts on neurones in the nerve cord and brain to inhibit the CA (ENGELMANN, 1964); (3) that since implantation of an egg case into the haemocoel does not inhibit the CA the stretched brood sac possibly liberates the inhibitory agent (ENGELMANN, 1968).

ENGELMANN'S (1964) principal objection to the mechanical inhibition theory was that no mechanoreceptors had been demonstrated that could be involved with inhibition of the CA during pregnancy in *Leucophaea*; also inhibition of the ovarian oöcytes after replacing the egg case with an artificial one inhibited normal feeding activity which affects resumption of activity of the CA (ENGELMANN and RAU, 1965).

I have re-examined this problem using *Eublaberus posticus* (Erich.). Reproduction of this blaberid is similar to other ovoviviparous cockroaches, but it is also capable of developing its oöcytes even when starved for 9 months (ROTH, 1968), thus eliminating the problem of a possible nutritional effect on the CA.

#### MATERIALS AND METHODS

*Eublaberus posticus* was reared and maintained on Purina Laboratory Chow, in an insectary at about 26°C and about 70% r.h. Drinking water was supplied in cotton-stoppered vials or test-tubes. All females were exposed to males within a day or two after emergence; females usually mate when white and teneral but mating may occur in older individuals (ROTH, 1968). The females were checked daily for spermatophores and if they had mated were isolated in beakers. The females used were in their first preoviposition and gestation cycles, except in experiments which required determination of the second preoviposition period.

Various experiments were performed at different times and over long periods and some fluctuation of insectary temperatures altered the length of gestation periods. Therefore, females for each series of experiments were kept for determinations of the time required for uterine eggs to hatch. Gestation periods varied from 55 to 58 days, 59 to 66 days, and 67 to 71 days. Oöcyte length was used as an indicator of corpus allatum activity (Roth and Stay, 1962b). The lengths of basal oöcytes of females carrying oöthecae were:

Days pregnant	Length (mm) of oöcytes		
	Range	Mean $\pm$ S.E.	N
55-58	1.29-2.55	1.82 $\pm$ 0.06	35
59-66	1.57-2.60	1.91 $\pm$ 0.05	28

The length of the basal oöcytes of females less than 24 hr after parturition (gestation 59–66 days) ranged from 1.53 to 3.10 mm, with a mean of  $2.04 \pm 0.06$  mm ( $N = 29$ ); only 1 female had oöcytes 3.1 mm long. Females with gestation periods of 66 to 71 days had oöcytes  $1.82 \pm 0.02$  mm long (range = 1.43 to 2.72 mm;  $N = 16$ ), less than 24 hr after parturition.

Oöcytes 2 mm or more in length have yolk in them. Except for a few cases which occur shortly after parturition, the oöcytes of *Eublaberus* contain little or no yolk at the time of parturition. After parturition the oöcytes grow rapidly. The experimental insects used to determine precocious oöcyte development following various treatments were about 2 to 4 weeks prior to parturition and were examined at about the time of or less than 24 hr after parturition or several days before parturition would normally have occurred. Basal oöcytes 3 mm or more long were considered to have developed precociously.

Carbon dioxide anaesthesia was used for all operations. For uterine implants the uteri and oöthecae of donor females were removed but left attached to their terminal sclerites (Fig. 2). A window was cut in tergites 5 and 6 to the right of the midline of host females (Fig. 1) and the donor uteri with oöthecae were implanted with the terminal sclerites protruding (Fig. 5). For implanting stretched uteri, the oöthecae of donor females were removed. The empty uteri were stretched with glass 'oöthecae', tied closed near the ovipositor, removed, and implanted into the abdomens of host females. Nerve cords were transected by making incisions ventrally at different levels and cutting between ganglionic connections. A small amount of aureomycin powder was used in all surgical experiments and the wounds were sealed with paraffin.

Histological sections were made of the uterus, fixed in Bouin's, cut at  $7 \mu$  and stained with Harris' haematoxylin and fast green, or eosin. Scanning electron micrographs were made of the everted uterus after coating with palladium-gold *in vacuo*.

#### RESULTS AND DISCUSSION

After removing the oötheca, the oöcytes of *Eublaberus* develop prematurely whether or not the female is fed. Insertion of a plastic or false oötheca into the uterus inhibits ovarian development (Table 1). ENGELMANN (1964) stated that '... females of *Leucophaea* carrying "paraffin egg cases" generally ate less than those from which the egg cases had only been removed. The few animals which ate like the controls, even though they were carrying artificial egg cases, deposited yolk in their oöcytes comparable to the control animals' (unpublished data). The number of animals was not indicated, but in a later paper ENGELMANN and RAU (1965) observed the effect of an artificial oötheca on 3 animals and found that feeding was invariably inhibited for 25 days. But 1 female then began to consume food like females that had their oöthecae removed and yolk was deposited in the oöcytes during the following days, in spite of the presence of an artificial egg case. ENGELMANN and RAU (1965) removed the oöthecae from 12 females of *Leucophaea* and found that 6 (50 per cent) of the insects did not have yolk in their oöcytes at

TABLE 1—OÖCYTE DEVELOPMENT FOLLOWING VARIOUS TREATMENTS AFTER REMOVING THE OÖTHECA OF *E. posticus*

Treatment following removal of oötheca*	Days pregnant when treated	Days after treatment when examined	Number (%) of females with ovaries					N
			Pre-cociously developed	Oöcytes†	Not Precociously developed	Oöcytes		
Fed	40	14	26 (63)	5.76 ± 0.11	15 (37)	1.81 ± 0.05	41	
	43	16-19	22 (71)	6.05‡ ± 0.27	9 (29)	2.07 ± 0.16	31	
Starved	40	14	18 (69)	5.84 ± 0.14	8 (31)	1.84 ± 0.14	26	
	43	17	23 (72)	5.96§ ± 0.26	9 (28)	2.33 ± 0.12	32	
Plastic 'oötheca' inserted in uterus (fed)	40	14	0		26 (100)	1.77 ± 0.05	26	
	43	17	6 (18)	3.58 ± 0.25	27 (82)	2.35 ± 0.02	33	

\* Gestation period ranged from 58 to 66 days.

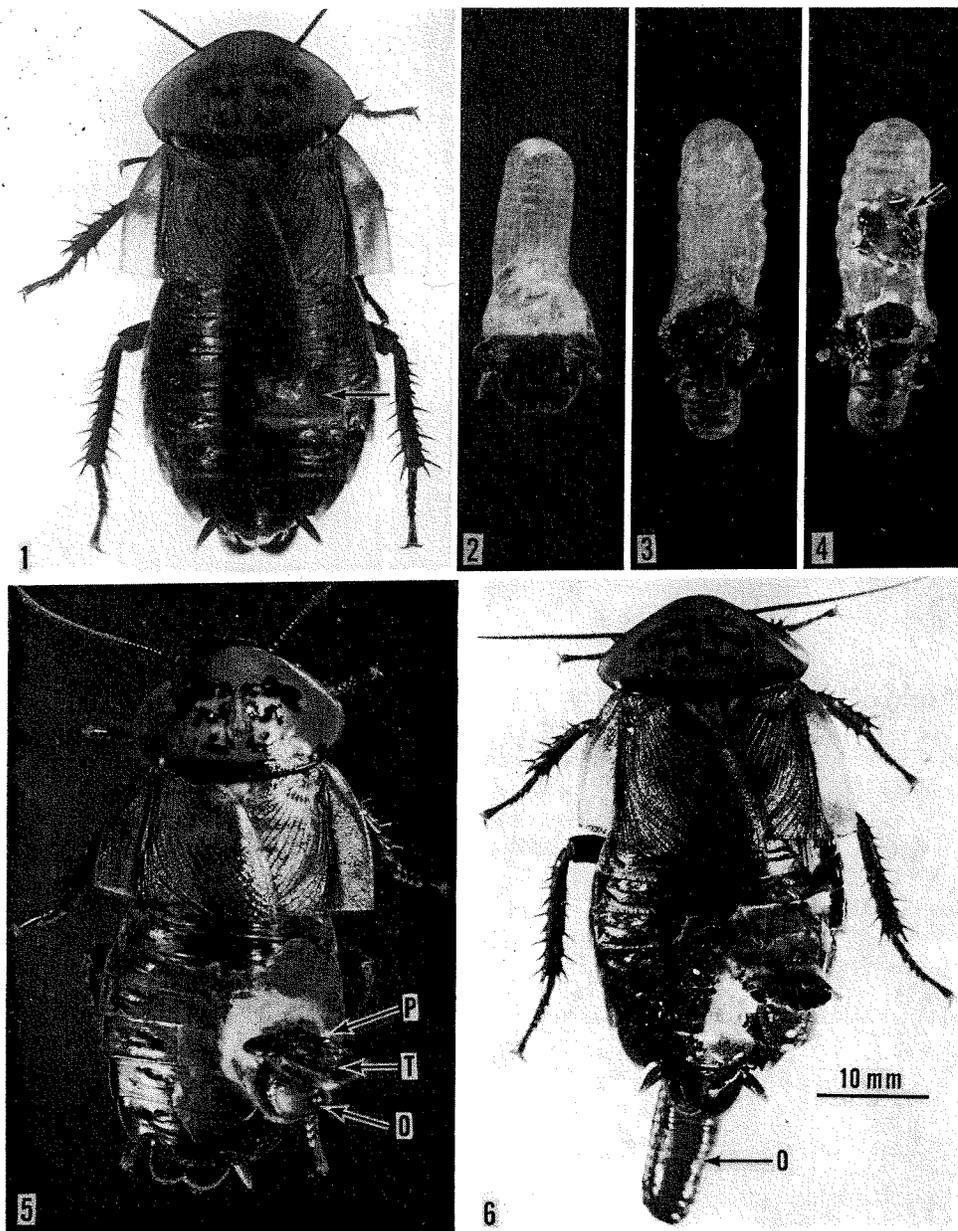
† Length in mm ± S.E. Females which had oviposited were counted as having oöcytes 7 mm long.

‡ Five females had oviposited.

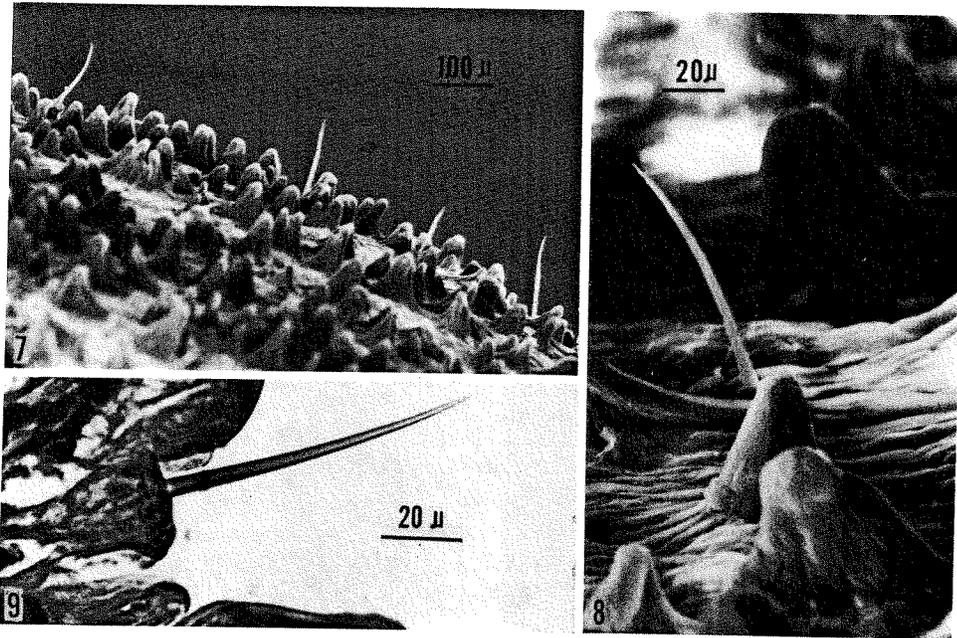
§ Two females had oviposited.

the end of the experimental period. In *Eublaberus* removing the oötheca results in precocious oöcyte development in 63 to 74 per cent of the females (Tables 1, 2, 3). Those females which did not oviposit precociously ovulated in about the same time as normal females that gave birth (Table 3). It is possible that in some females nerves to the sixth ganglion are damaged in removing the oötheca and this may account for the oöcytes not developing prematurely. Considering the variation one finds after removing the oötheca, it is dangerous and unwarranted to draw conclusions about the effect of an artificial oötheca on oöcyte development of only 3 animals. Also, an artificial oötheca will not inhibit oöcyte growth indefinitely presumably because of adaptation of mechanoreceptors or the central nervous system.

Implantation into the abdomen of either a uterus stretched with an artificial oötheca or a uterus containing a real oötheca did not inhibit precocious oöcyte development in host females whose oöthecae had been removed (Table 2). Females with oöthecae removed and with implanted uteri containing oöthecae oviposited in about the same period of time as females who only had their oöthecae removed (Table 3). The time between the first and second ovipositions of all these females was about 3 weeks less than untreated controls (Table 3). Many of the eggs in the implanted uteri grew and stretched the uterus (Fig. 3); the eggs which died were usually in the posterior part of the oötheca and were those which extended outside the abdomen of the host female. The terminal segments of the implant are not held tightly together so that exposed eggs (Fig. 5) desiccate. The fact that an



FIGS. 1-6. Uterine implantation in *E. posticus*. (1) Host female (wings cut off at level of the abdomen) with a window (arrow) cut in the tergites to receive the implant; oötheca of host removed at 36.5 days of pregnancy. (2) Donor uterus, attached to the terminal segments, containing an oötheca less than 1 day old. (3-4) Uterus that had been implanted for 50 days. Eggs that had protruded from beyond the terminal segments died whereas those within the abdominal cavity developed. The intestinal tracts of 2 embryos are visible through the uterus in Fig. 4. 'Melanotic' lesions (arrow) on the ventral surface of the uterus shown in Fig. 3. (5) Uterus and oötheca (from Fig. 2) implanted into the abdomen of the female shown in Fig. 1. P, paraffin seal; T, terminal tergite (supra-anal plate) of implant; O, terminal eggs of oötheca exposed between the supra-anal and sub-genital plates of the implant. (6) Female ovipositing 29 days after having a uterus and oötheca implanted into its abdomen. O, oötheca of host being retracted into the uterus. (All to same scale shown in Fig. 6.)



FIGS. 7-9. Sensilla trichodea in the uterus of *Eublaberus posticus*. (7-8) Scanning electron micrographs showing the mechanoreceptors among the uterine papillae. (9) Histological section showing a trichoid sensillum.

TABLE 2—OÖCYTE DEVELOPMENT FOLLOWING UTERINE IMPLANTS IN *E. posticus*

Treatment following removal of oötheca	Days pregnant when treated	Days after treatment when examined	Number (%) females with ovaries				N
			Pre-cociously developed	Oöcytes*	Not Precociously developed	Oöcytes	
Stretched uterus implanted into abdomen†	43-44‡	13	9 (90)	4.44 ± 0.30	1 (10)	2.27	10
Sham operated	43-44	12	8 (80)	4.49 ± 0.16	2 (20)	1.60 ± 0.07	10
Uterus plus oötheca implant§	36-37	17	25 (74)	4.08 ± 0.12	9 (26)	2.36 ± 0.16	34
Oötheca removed only	36-37	17	18 (64)	5.11 ± 0.19	10 (36)	1.65 ± 0.10	28

\* Length in mm ± S.E.

† Uteri were taken from females 29 to 30 and 46 to 52 days of gestation. Histological sections of two implanted uteri at the end of the experiment showed them to be normal.

‡ Oöcytes of females at 43-44 days of gestation are 1.45 ± 0.02 mm ( $N = 5$ ).

§ Donor uteri and oöthecae were from females 0 to 2 days after oviposition.

|| Oöcytes of females at 36-37 days of gestation are 1.29 ± 0.02 mm ( $N = 10$ ).

TABLE 3—OVIPOSITION BY *E. posticus* FOLLOWING UTERINE (PLUS OÖTHECA) IMPLANTS

Conditions	N	Number (%) which oviposited	Days between first and second ovipositions	
			Range	$\bar{X} \pm S.E.$
Untreated*	25	25 (100)	83-92	87.0 ± 0.5
Oötheca removed† only	46	34 (74)‡	61-78.5	65.5 ± 0.6
		12 (26)	83-94.5	89.8 ± 0.9
Oötheca removed† and a uterus and oötheca implanted into abdomen	36	35 (97)	63-74	67.4 ± 0.5§

\* Gestation varied between 66 and 71.5 days ( $\bar{X} = 69.1 \pm 0.2$  days). The second preoviposition period (after parturition) ranged between 14.5 and 22.5 days ( $\bar{X} = 17.9 \pm 0.5$  days).

† Removed at 36 to 37 days of pregnancy.

‡ The females are separated into two groups: Those in which the oöcytes developed precociously (74 per cent) and those which did not (26 per cent).

§ Females oviposited 26.5 to 37.5 days ( $\bar{X} = 31.3 \pm 0.5$  days) after uterine implantation. One did not oviposit and its oöcytes were undergoing resorption 49 days after implantation.

implanted uterus with developing eggs does not prevent precocious oöcyte development in the host argues against Englemann's hypothesis that a hormone possibly originating from the stretched uterus during pregnancy inhibits oöcyte development.

Although many of the embryos in the implanted uterus become well developed (showing pigmented eye spots, appendages) they usually died before reaching maturity. This may be due to the fact that the implanted uterus is denervated (see below).

It was not uncommon for the implanted uteri to develop black necrotic tumour-like lesions on the outer ventral surface. On the inner surface of the uterus the blackened regions conformed to the contour of the wall, probably due to pressure of the oötheca. However, on the outside wall, in contact with the haemolymph, the lesion was irregular, thickened, and protruded above the uterine surface. Only 15 of the 36 females with implanted uteri were examined for lesions, which were present in every case. These necrotic lesions were never seen on the uteri of the host females.

Dr. Ronald Taylor examined sections of a uterine lesion and commented (personal communication) as follows: "There appears to be very little blood-cell involvement in your lesions, although there wouldn't necessarily be any if the lesion has had time to heal. There is evidence of recent blood-cell clotting around the "melanotic" area. Some bacteria (grayish encapsulated areas) and a damaged trachea also appear to be involved. There doesn't appear to be any metaplastic, hyperplastic, or neoplastic response on the part of the uterine epithelium or any other tissue, although the lesion does appear to be confined to the epithelial region and its closely associated muscles. The uterine intima appears not to be broken although it has darkened above the lesion. I have seen this happen to other cuticle that normally doesn't darken (hindgut) which was adjacent to disintegrating blood-cells. Possibly polyphenol oxidases or quinones or other substances involved in melanization are released from the dying blood cells and diffuse into the cuticle where they are normally not present and bring about the resultant darkening. . . . Also disruption of the normal metabolism of the underlying epithelium might somehow be involved in the abnormal darkening."

Midgut implants become 'tumorous' (MATZ, 1961), and tumour-like lesions are induced in the alimentary canal after severing the recurrent nerve. TAYLOR (1969) discusses the various hypotheses to explain why tumour-like lesions occur following denervation. He believes these to be inflammatory lesions resulting from an abnormally engorged foregut. Stagnation occurs throughout the alimentary canal allowing micro-organisms which infect the gut epithelium to proliferate. Blood cells infiltrate the injured areas and establish inflammatory foci. The uterus of pregnant *Eublaberus* is 'engorged' with growing eggs, but this engorgement is normal and cannot be compared with alimentary canal blockage following denervation. Since the implanted uterus is denervated, the elimination of nervous stimuli may have something to do with the formation of necrotic uterine lesions. Severing the nerves posterior to the last ganglion induces necrotic lesions in the uterus of

some females, but the lesions do not occur if the connectives are transected between the fifth and sixth ganglia (see below). Thus, nervous connexions to the uterus from the last abdominal ganglion prevents lesion induction even though this ganglion is isolated from the remainder of the central nervous system.

ENGELMANN (1964) found that the effect of transecting the nerve cord in pregnant *Leucophaea* on oöcyte development varied with the site of transection. Cutting the cord between the fifth and sixth ganglia resulted in only 33 per cent of the females whose oöcytes developed precociously, versus 88 per cent for females whose cord was severed between the second and third ganglia. None of the females which had various or all the nerves posterior to the sixth ganglion severed developed oöcytes. I initially performed this experiment with *Nauphoeta cinerea* (Oliv.) and obtained somewhat similar results (Table 4). However, some females which aborted their oöthecae after severing the cord between the fifth and sixth ganglia or cutting the nerves posterior to the sixth ganglion also failed to develop their oöcytes. Removing the oötheca and then cutting the cord between ganglia five and six gave development of the oöcytes in only a small percentage of pregnant females (Table 4).

TABLE 4—OÖCYTE DEVELOPMENT FOLLOWING NERVE CORD TRANSECTION AT DIFFERENT LEVELS OF *N. cinerea*

Site of transection (between ganglia)*	N	Number (%) of females with yolk in basal oöcytes†
Oötheca left in uterus		
Second and third, third and fourth, fourth and fifth	21	17 (81)
Fifth and sixth	13	0
Nerves posterior to sixth	7	0
Sham operated	8	0
Oötheca removed from uterus		
Third and fourth	13	11 (85)
Fifth and sixth	16	2 (13)
Unoperated	20	17 (85)

\* Females were 23 to 24 days pregnant. Gestation lasts 35 to 50 days.

† Examined 12 days after the operations.

Transecting the nerve cord between various ganglia of *Eublaberus* resulted in a marked decrease in the number of pregnant females which developed their oöcytes only when the nerves posterior to the last abdominal ganglion were severed. However, as in *Nauphoeta* the result was essentially the same if the oötheca was first removed and all of the nerves from the last ganglion were then severed (Table 5). Engelmann's control for these experiments in *Leucophaea* was inadequate for he failed to determine what the effect on oöcyte development would be

following severing the cord at different levels after the female's oötheca was removed (thus removing the inhibition). Although cutting all the nerves posterior to the last ganglion of pregnant females resulted in only 22 per cent showing precocious oöcyte development, this number was increased to 70 per cent when the

TABLE 5—OÖCYTE DEVELOPMENT FOLLOWING NERVE CORD TRANSECTION AT DIFFERENT LEVELS OF *E. posticus*

Site of transection (between ganglia)*	Days after operation	Number (%) of females with oöcytes				N
		Precociously developed	Oöcyte length (mm) ± S.E.	Not precociously developed	Oöcyte length (mm) ± S.E.	
Metathoracic and first abdominal: oötheca intact	16-17	14 (82)	5.61 ± 0.22	3 (18)	1.82 ± 0.08	17
Third and fourth						
Oötheca intact	13-19	10 (100)	5.92 ± 0.04	0	—	10
Oötheca removed†	17-18	6 (100)	6.52 ± 0.27	0	—	6
Fourth and fifth						
Oötheca intact	14-19	30 (94)	5.64 ± 0.18	2 (6)	2.45 ± 0.05	32
Oötheca removed	16-18	10 (100)	6.27 ± 0.13	0	—	10
Fifth and sixth						
Oötheca intact	17-19	17 (85)	5.53 ± 0.22	3 (15)	2.13 ± 0.26	20
Oötheca removed	17-19	12 (100)	6.34 ± 0.01	0	—	12
Nerves posterior to sixth						
Oötheca intact	17-19	9 (22)	4.61 ± 0.38	31 (78)	2.03 ± 0.07	40
Oötheca removed	17-19	7 (27)	4.96 ± 0.55	19 (73)	1.98 ± 0.03	26
Oötheca removed‡	17	10 (29)	3.94 ± 0.28	24 (71)	2.17 ± 0.10	34
Anterior and posterior to sixth						
Oötheca intact§	14	30 (70)	4.48 ± 0.15	13 (30)	2.19 ± 0.12	43
Sham operations						
Oötheca intact	17-19	0	—	13 (100)	2.08 ± 0.12	13

\* Gestation period 59 to 66 days. Except for one experiment (footnote §) females were 43 days pregnant at the time of the operations.

† Transections were made just after removing the oötheca except for experiment footnote ‡.

‡ Nerves cut 13 to 28 hr after removing the oötheca.

§ Females were 40 days pregnant when operated upon. The sixth ganglion was either removed ( $N = 23$ ) or the completely isolated ganglion was left in the haemocoel ( $N = 20$ ). The data are combined because there was no difference in the results from both groups.

sixth ganglion was severed completely and isolated, and was either removed or left in the abdomen (Table 5). These results stress the importance of the last abdominal ganglion in inhibiting the corpora allata. The fact that cutting the nerves posterior to the last ganglion prevented precocious oöcyte development in most females

whose oöthecae were removed suggests that disinhibition (i.e. an empty uterus after removing the oötheca several weeks before parturition) results in signals transmitted to the last abdominal ganglion which are necessary for reactivation of the CA. This possible message from an empty uterus is less crucial towards the end of gestation. In certain Blaberidae (e.g. *Nauphoeta cinerea*, *Diploptera punctata* (Eschscholtz), and *Eublaberus posticus*) yolk may be deposited in the basal oöcytes a day or more before parturition (ENGELMANN, 1959; ROTH and STAY, 1961, 1962b; ROTH, 1964b). The CA become activated before the female gives birth in spite of the presence of the oötheca. This may be due to gradual adaptation of uterine receptors or the central nervous system so that the last abdominal ganglion loses its ability to inhibit the CA towards the end of gestation.

The results of one experiment in which the uterus was removed may not support the hypothesis that a signal from an "empty" uterus is transmitted to the last abdominal ganglion. The oöthecae were removed from 22 females 40 days after oviposition. The everted uterus was tied closed below the ovipositors and then cut and removed. Fourteen days later 18 (82 per cent) had large oöcytes ( $5.96 \pm 0.12$  mm) indicative of precocious development; 4 (18 per cent) females had small oöcytes ( $1.67 \pm 0.07$  mm). It seems likely that with the above operation the uterine nerves were severed, but if they were they were cut close to the uterus; in other operations where nerves posterior to the sixth ganglion were cut they were severed close to the ganglion itself.

The following experiment was performed to determine if severing the nerves posterior to the last abdominal ganglion, at about the end of the gestation period, would delay oöcyte maturation. The nerves from the last ganglion or the connectives between the fifth and sixth ganglia were severed in *Eublaberus* females 66 to 68 days after ovipositing. These females should have given birth from 1 to 3 days later but the operations prevented normal parturition. Therefore oöthecae were removed manually if the females failed to give birth at about the expected time. Fourteen (54 per cent) of the females with nerves posterior to the sixth ganglion severed gave birth and of these 11 had their uteri completely everted. Three females did not extrude the entire oötheca. The uteri were pushed back into the abdomen with forceps, but eversion occurred repeatedly and many of the females had their uteri everted at completion of the experiment. Eversion of the uterus did not occur when the nerve cord was severed between the fifth and sixth ganglia indicating that signals from the last abdominal ganglion maintain the uterus in its normal retracted state.

Unfortunately, these operated females also cannot oviposit normally (Table 6) so that determining oöcyte maturation by observing time of oviposition usually was not feasible. Females which did not oviposit were dissected a few days after they should have done so and their oöcytes were measured (Table 6). Cutting the cord between the fifth and sixth ganglia did not delay the second preoviposition period in those females which had oviposited (though abnormally), and the elapsed time between the first and second 'ovipositions' was essentially the same as in sham or unoperated females. The oöcytes of non-oviposited females were large and almost

mature. The females which had the nerves posterior to the sixth ganglion severed had somewhat smaller oöcytes than females with the cord severed between ganglia 5 and 6, in spite of the fact that the former were dissected a few days later giving their oöcytes more time to grow. This indicates a somewhat slower rate of development following severance of nerves from the uterus to the sixth ganglion in late stages of pregnancy, but the delay is not as marked as when the same operation is performed several weeks before parturition.

TABLE 6—OÖCYTE DEVELOPMENT FOLLOWING NERVE CORD TRANSECTION IN LATE PREGNANCY OF *E. posticus*

Site of transection*	N	Number (%) which oviposited	Days ( $\bar{X} \pm$ S.E.) to oviposit following operation	Days ( $\bar{X} \pm$ S.E.) between first and second ovipositions	Oöcyte length (mm) ( $\bar{X} \pm$ S.E.) of females which did not ovi- posit
Between fifth and sixth ganglia†	34	18 (53)‡	21.4 $\pm$ 0.4	88.7 $\pm$ 0.4	5.9 $\pm$ 0.4§
Nerves posterior to sixth ganglion	26	0	—	—	4.5 $\pm$ 0.3¶
Sham operated	10	10 (100)	22.6 $\pm$ 0.6	90.4 $\pm$ 0.4	—
Unoperated	25	25 (100)	—	87.0 $\pm$ 0.5	—

\* Females were 66 to 68 days pregnant at time of operation.

† Oöthecae were removed at 68.5 to 73.5 days of pregnancy ( $\bar{X} = 71.3 \pm 0.2$  days).

‡ Only 1 female oviposited normally. Of the 17 others 11 oviposited a few eggs abnormally arranged in the uterus or vestibulum and 6 had only a blob of colleterial gland secretion, without any eggs, in the vestibulum.

§ Based on 11 females (dissected 25 to 26 days after the operation) which had measurable oöcytes; 5 females had oöcytes which had matured but were being absorbed.

|| Females gave birth or oöthecae were removed 67 to 72.5 days of pregnancy ( $\bar{X} = 70.0 \pm 1.1$  days).

¶ Based on 16 females (dissected 27 to 31 days,  $\bar{X} = 28$  days) which had measurable oöcytes; 10 females had oöcytes which had matured but were being absorbed.

Sixteen females (62 per cent) whose nerves were severed posterior to the last abdominal ganglion had uterine lesions. Only 1 female (3 per cent) whose cord was severed between the fifth and sixth ganglion had uterine lesions.

Severing the nerves anterior and posterior to the last abdominal ganglion results in death of the uterine eggs in 65 to 85 per cent of the females (Table 7). Since the females were examined at about the end of the normal gestation period, this mortality was not due to the oötheca being retained longer than normal because parturition was interfered with. Oöcyte development was not correlated

with uterine egg mortality since a high percentage of females whose oöcytes did or did not develop had dead uterine eggs. The results suggest that nerve impulses from the last abdominal ganglion to the uterus are important for the survival of uterine embryos in late stages of development. This may explain why in uterine implants (see above) the embryos develop but die before they reach the parturition stage.

TABLE 7—EFFECT OF TRANSECTING THE NERVE CORD AT VARIOUS LEVELS ON SURVIVAL OF UTERINE EGGS OF *E. posticus*

Site of nerve cord transection (between ganglia)	Number (%) of females in which oöcytes were Developed and uterine eggs were			Undeveloped and uterine eggs were		
	Dead	Alive	N	Dead	Alive	N
Metathoracic and first abdominal	0	14 (100)	14	0	3 (100)	3
Third and fourth	0	10 (100)	10	—	—	—
Fourth and fifth	1 (3)	29 (97)	30	0	2 (100)	2
Fifth and sixth	2 (12)	15 (88)	17	—	3 (100)	3
Nerves posterior to sixth	6 (67)	3 (33)	9	20 (65)	11 (35)	31
Sixth ganglion removed or com- pletely severed and left in haemocoel	21 (70)	9 (30)	30	11 (85)	2 (15)	13

ENGELMANN (1964) concluded that there were no mechanoreceptors on the genitalia or abdominal musculature that could be involved with detecting the oötheca in the uterus related to inhibition of the CA. However, BROUSSE-GAURY (1971a, b) has described mechanoreceptors (sensilla trichodea) in the uteri of *Leucophaea*, *Blaberus*, *Nauphoeta*, and *Gromphadorhina*. She suggested that in these genera inhibition of the CA during pregnancy is due to nervous stimuli resulting from deflection of uterine trichoid sensilla by the oötheca in the uterus. Similar mechanoreceptors are found in the uterus of *Eublaberus* (Figs. 7–9) and the present experiments strengthen the hypothesis that mechanical stimuli from these uterine receptors are transmitted to the last abdominal ganglion. The sixth abdominal ganglion may well '... play the role of a primary integrator' for the stimuli reaching it from the uterus, as has been suggested for mating stimuli in *Blaberus craniifer* Burm. (GRILLOU, 1971). How this information reaches the brain is unknown.

BESSÉ (1967) has correlated cyclical activity of Type A neurosecretory cells in certain nerve cord ganglia of *Leucophaea maderae* with the ovarian cycle. During the first preoviposition period when the oöcytes are accumulating yolk, the Type A cells discharge their secretion. About 10 days after ovulation, neurosecretion begins to accumulate in the cells and the material is stored during gestation. About 48 hr after parturition most of the neurosecretory material is liberated, and this is followed by an increase in neurosecretion in the cells until a level is reached

at the second incubation period corresponding to that of the first gestation period. These findings suggest that neurosecretion which is liberated during the first and the start of the second vitellogenesis contribute in some way to oöcyte maturation. BROUSSE-GAURY (1971c) has shown that the activity of neurosecretory cells is controlled by sensory impulses; the endocrine glands can be controlled by means of neurosecretory material along neuroendocrine pathways which are influenced by exteroceptive stimuli.

It is possible that inhibitory and activating signals are transmitted to the last abdominal ganglion which in some way then controls the activity of the ganglionic neurosecretory cells whose neurosecretion in turn may control the activity of the CA. In *Leucophaea*, Type A cells are found in the thoracic, and abdominal ganglia 1 to 3 only; none are present in ganglia 4 to 6 (BESSÉ, 1967). Nothing is known of the distribution of neurosecretory cells in *Nauphoeta* or *Eublabeus* and species differences could account for somewhat variable results following transecting the nerve cord at different levels.

*Acknowledgements*—I thank Drs. BARBARA RAISEBECK and JACK GINGRICH for making the histological preparations of the uterus and Mr. SAMUEL COHEN for taking the photographs and the scanning electron micrographs. I am also grateful to Dr. RONALD TAYLOR, California State College, for his comments on the uterine lesions.

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