JOUR. FAC. SCI., SHINSHU UNIV., Vol. 7, pp. 13–28, June 1972

The Effect of the Electrical Stimulation on the Termination of the Pupal Diapause in the Giant Silkworm, Samia cynthia pryeri Butler

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(received April 30)

Abstract

The effect of the electrical stimulation on the termination of the diapause of the pupa was investigated in a giant silkmoth, *Samia cynthia pryeri* (*Philosamia cynthia cynthia*). It has been known in this insect that for the development of the pupa the long period of exposure to the low temperatures before the incubation is required. It has also known that the diapause of the pupa is under the direct control of the activity of the prothoracic glands and then the activity of the prothoracic glands itself is governd by the activity of the neurosecretory cells of the pupal brain. The following results were given from these experiments. (1) The period between the beginning of the incubation and the activation of the neurosecretory cells of the chilled pupa was shortened by the electric stimulation. (2) The brain of the non-chilled pupa was brought to such an activated state by the electrical stimulation as the diapausing non-chilled pupa transplanted the brain of the stimulated pupa initiated the further development. In these experiments a possibility of the direct activation of the prothoracic glands to the electric stimulation seems to be excluded from the fact that no effect was brought out by the stimulation of the pupa extirpated of the brain before the stimulation. A relation was found between the electrical activity of the pupa and the results whether the diapausing pupa transplanted the brain which was obtained from the pupa recorded the electrical activity developed further or not. Therefore, it seems to be probable from these results that the activation of the secretion of the pupal brain is induced by the nerve impulses.

Introduction

There has been clarified that the pupal diapause of a giant silkmoth, *Samia*

cynthia pryeri, (*Philosamia cynthia cynthia*), is induced and kept by the inactivation of the prothoracic glands of the pupa, and it is terminated by the activation of the prothoracic glands. The activity of the prothoracic glands itself is under control of the secretory activity of the brain, supraoesophageal ganglia, of pupa. (FUKUDA, S. '59). These events in the diapause of cynthia pupa were quite in accordance with those events in the diapause of cecropia pupa clarified by C. M. WILLIAMS. ('46b, '47, '52).

It has been a well known fact that for the further development of the diapausing pupa, the long exposure of the pupa to the low temperatures before the incubation is required. According to VAN DER KLOOT a spontaneous electrical activity of the diapausing cecropia pupal brain was kept inactive, while it became active before the occurrence of the initial characteristics of the development when the chilled pupa was incubated. ('55). He reported also the facts that acetylcholine-choline esterase activity decreased in the non-chilled pupa, acetylcholine of the brain increased in the chilled pupa and acetylcholine-cholinesterase activity of the brain increased correspond to the occurrence of the spontaneous electrical activity of the brain in the incubated chilled pupa. It was reported in cynthia pupa that the electrically inactive brain was inactive in secretory activity on the one hand, the electrically active brain was active in secretory function on the other hand, the increase of the spontaneous electrical activity occurred before the increase of the secretory activity. (KOENUMA, A. '68). These facts suggest the possibility of the following sequential events for the termination of the diapause. Any nerve function is activated by the thermal stimuli of the incubation in the brain of the chilled pupa, then the neurosecretory cells of the brain begins to secrete the brain hormone to be activated by a definite threshold stimulus of the nerve impulses, finally the activation of the prothoracic glands occurs in response to the brain hormone, thus the pupa begins to develop in response to the prothoracic gland hormone. If such a mechanism really existed, the development of the diapausing pupa might be accelerated by the repeating stimuli of the weak electric current of the head region of the pupa. In present study, some experiments were carried out to investigate the possibility above mentioned.

Material and Methods

The pupae used for these experiments were those collected near Matsumoto city in early October 1970. They were stored in an incubator at 26°C until their use in the experiments. On late August 1971, 56 pupae which were the rest of the pupae used for experiments were survived in diapausing, while 2 developed to imagos. The intact pupae stored in an incubator were used as the non-chilled pupae, and the pupae which were chilled for over 8 weeks in a refrigerator at

5°C before the experiment were used as the chilled pupae. The pupae were divided into 8 groups each of which consisted of 7 pupae, and the following experiments were carried out.

(A) Non-chilled pupa was stimulated electrically through the pair of platinum electrodes implanted into the head of the pupa and the dorsal region of the 4-th abdominal segment of the pupa. The pupa was stimulated repeatedly once per one second for 3 days with electrical pulses each of which was 1 Volt 10 ms. and caudal to head direction.

(B) The brain isolated in the experiment (A) was transplanted into the dorsal region of the 4-th abdominal segment of the non-chilled pupa.

(C) The same experiment as the experiment (A) was carried out in the chilled pupa simultaneously its beginning of incubation.

(D) The same experiment as the experiment (C) was carried out in the decerebrated chilled pupa.

(E) The effect of the decerebration which was carried out 5 days after the incubation of the chilled pupa.

(F) The brain isolated in the experiment (D) was transplanted to the non-chilled pupa.

(G) The brain isolated in the experiment (C) was transplanted to the non-chilled pupa.

(H) The brain isolated in the experiment (E) was transplanted to the non-chilled pupa.

The spontaneous electrical activity was recorded just after the finishing the stimulation (3 days after the beginning of the experiment) and just before the extirpation of the brain (5 days after the beginning of the experiment) through the implanted platinum electrodes in the every pupa of the experiment (A) and (C) and in some of the pupae of the experiment (E). In the recording of the spontaneous electrical activity of the pupa, a set of the devices was used, where an amplifier of Nihon Kohden KK. model AVB-2, model VC-7 osciloscorp system and model EPR-2 electronic polyrecorder of TOA Electronics Ltd. consist of a set. These were connected with condensors each other.

Every pupa was reared in an incubator at 26°C about 60% humidity. To avoid the pupa to die by drying, sterized distilled water was injected into the pupa in sometimes. The stimuli were supplyed from an electronic stimulator of Nihon Kohden KK. model MSE-3.

Results

1. *The effect of the electrical stimulation on the development of the decerebrated pupa.*

Table 1 development of the decerebrated pupae

expr.	No. of pupae	chilling	period of decerebration	stimulation	survival	development ⫲	⫲	+	−
			Day		Days				
A	7	−	5	+	46–97	0	0	0	7
C	7	+	5	+	45–98	0	5	0	2
D	7	+	0	+	45–126	0	0	0	7
E	7	+	5	−	45–65	0	0	0	7

The effect of the electrical stimulation on the pupa of which brain was removed 5 days after beginning of the incubation on the development was present in Table 1. In this table A, C, D and E represent the experiment (A), (C), (D) and (E) respectively, and the symbols ⫲, ⫲, + and − represent the various degrees of the development, where the pupa developed to a complete moth, the complete moth was not formed but skin, antenna and legs were formed, skin was formed without antenna and leg formation and any development of the pupa did not occur respectively. As it was clearly shown in this table, the development did not occur in the non-chilled pupa. In the chilled pupae, however, the development did not occur in the pupae without stimulation on the one hand, various degrees of the development occurred in the pupae with stimulation on the other hand. None of the pupae extirpated of brain before the stimulation developed further.

2. *The effect of the electrical stimulation of the donor pupa on the development of the non-chilled pupa which was transplanted the former brain.*

The results of the experiment (B), (F), (G) and (H) were present in Table 2. It is clearly shown in this table that about half of the pupae developed in various degree in every case. Particularly, it seems quite important that the pupa developed not only in some degree but also into complete imago when the brain of the electrically stimulated non-chilled pupa was transplanted to the non-chilled pupa. A moth developed in the experiment (B) in which the brain of the electrically stimulated non-chilled pupa was transplanted to the non-chilled pupa is present in Fig. 1.

Table 2 development of the brain-transplanted non-chilling pupae

expr.	condition of the transplanted brain				survival	development ⫲	⫲	+	−
	No. of pupae	chilling	period of decerebration	stimulation					
			Day		Days				
B	7	−	5	+	45–111	1	0	3	3
F	7	+	0	−	35–125	1	1	2	3
G	7	+	5	+	60–106	3	0	1	3
H	7	+	5	−	45–125	0	1	3	3

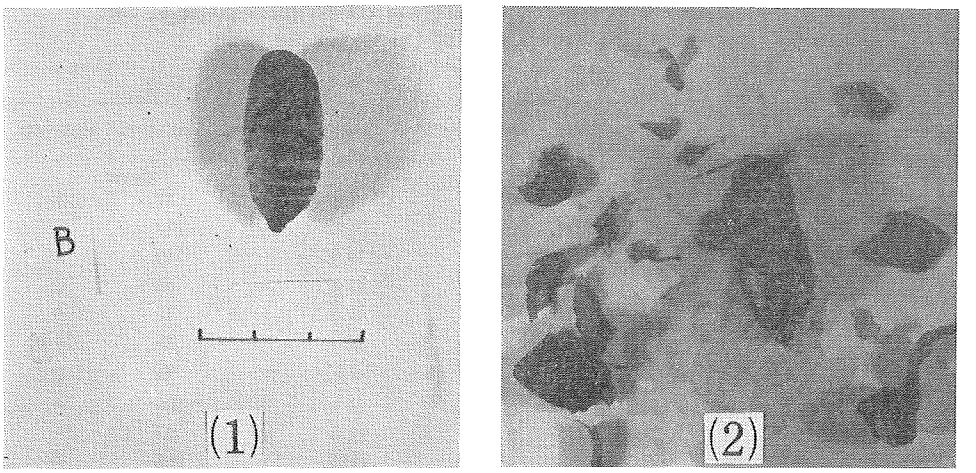

Fig. 1 development of the pupa transplanted the brain of the stimulated non-chilled pupa
(1) diapausing pupa (2) after development

Table 3 development of the donor pupae and the recipient pupae in the transplantation experiments of the non-stimulated brain

donor					recipient			
pupa	chilling	period of decerebration	survival	develop.	pupa	chilling	survival	develop.
		day	days				days	
D 1	+	0	108	−	F 1	−	126	++
D 2	+	0	126	−	F 2	−	35	−
D 3	+	0	66	−	F 3	−	125	+
D 4	+	0	104	−	F 4	−	69	+++
D 5	+	0	45	−	F 5	−	45	+
D 6	+	0	45	−	F 6	−	93	−
D 7	+	0	45	−	F 7	−	93	−
E 1	+	5	45	−	H 1	−	124	−
E 2	+	5	60	−	H 2	−	124	+
E 3	+	5	60	−	H 3	−	125	−
E 4	+	5	65	−	H 4	−	125	+
E 5	+	5	45	−	H 5	−	45	+
E 6	+	5	45	−	H 6	−	107	++
E 7	+	5	45	−	H 7	−	107	−

It was difficult to find out the remarkable difference among the results of the experiment (F), (G) and (H). Therefore, it may be probable to consider that the transplanted brain of the electrically stimulated pupa brought no inhibitory effect on the development of the recipient pupa.

Table 3 indicated the development of the donor pupa and of the recipient pupa in the transplantation experiments in which the brain of the chilled pupa without stimulation isolated at the 5-day incubation was transplanted to the non-

Table 4 development of the donor pupae and the recipient pupae in the transplantation experiments of the stimulated brain

donor					recipient			
pupa	chilling	period of decerebrated	survival	develop.	pupa	chilling	survival	develop.
		day	days				days	
A 1	−	5	93	−	B 1	−	47	++
A 2	−	5	93	−	B 2	−	111	−
A 3	−	5	97	−	B 3	−	111	−
A 4	−	5	97	−	B 4	−	83	+
A 5	−	5	46	−	B 5	−	45	+
A 6	−	5	83	−	B 6	−	45	+
A 7	−	5	89	−	B 7	−	45	−
C 1	+	5	77	−	G 1	−	106	−
C 2	+	5	98	−	G 2	−	93	−
C 3	+	5	98	++	G 3	−	106	++
C 4	+	5	98	++	G 4	−	106	−
C 5	+	5	45	++	G 5	−	60	++
C 6	+	5	45	++	G 6	−	92	++
C 7	+	5	45	++	G 7	−	92	++

chilled pupa. In these cases no pupa extirpated of the brain developed. This fact indicates that the extirpation of the brain was complete, the brain remained still inactive at removal time. In both cases, in which the non-chilled pupa was transplanted the brain isolated before the incubation and it was transplanted the brain isolated at 5-day incubation, the half of the pupae developed.

Table 4 indicated the development of the donor pupa and the recipient pupa when the non-chilled diapausing pupa was transplanted the brain which was isolated from the non-chilled pupa with 3-day stimulation at 5-day of incubation. This table indicates also the development of the donor pupa and the recipient pupa when the non-chilled diapausing pupa was transplanted the brain which was isolated from the chilled pupa with 3-day stimulation at 5-day of incubation. In this table, it was crearly shown that none of the non-chilled pupa extirpated of the brain at 5-day incubation developed in spite of the stimulation. This indicates that the brain remained still inactive to secrete the hormone at 5-day of incubation.

In the case in which the non-chilled pupa was transplanted the brain of the stimulated non-chilled pupa, not only the somewhat development of the pupa occurred in about a half of the pupae but also a complete moth was formed in one pupa.

Somewhat development of the pupa occurred in 4 pupae of the 7 stimulated chilled pupae extirpated of thir brains. About a half of the non-chilled pupae which were transplanted the brain obtained from the above mentioned pupae

Table 5 effect of the electrical stimulation of the pupal brain on the development

donor				recipient		
pupa	stimulation	survival	develop.	pupa	survival	develop.
		days			days	
C 1	+	77	−	G 1	106	−
C 2	+	98	−	G 2	93	−
C 3	+	98	++	G 3	106	++
C 4	+	98	++	G 4	106	−
C 5	+	45	++	G 5	60	++
C 6	+	45	++	G 6	92	++
C 7	+	45	++	G 7	92	++
E 1	−	45	−	H 1	124	−
E 2	−	60	−	H 2	124	+
E 3	−	60	−	H 3	125	−
E 4	−	65	−	H 4	125	+
E 5	−	45	−	H 5	45	+
E 6	−	45	−	H 6	107	++
E 7	−	45	−	H 7	107	−

developed in some degree. Of those, the pupa developed to a complete moth in one case.

3. *The effects of the electrical stimulation on the extirpation experiments and the transplantation experiments of the pupal brain.*

The effect of the extirpation of the brain of the stimulated chilled pupa at 5-day of incubation and the effect of the transplantation of thus isolated brain to the non-chilled pupa on the development were indicated in Table 5. In this table, the effect of the extirpation of the brain of the chilled pupa without stimulation and the effect of the transplantation of the brain isolated from the chilled pupa without stimulation to the non-chilled pupa on the development were also indicated. The brain was transplanted like the following manner as the brain of the C_1 pupa was transplanted to the G_1 pupa and C_2 to G_2. As clearly shown in this table, none of the decerebrated chilled pupa without stimulation developed. In contrast to those results, some pupae developed fairly regardless to the decerebration when the pupae were stimulated. The transplantation of the brain of the stimulated pupa was more effective to the development of the recipient pupa than that of the brain of the pupa without stimulation. These results suggest that the brain of the chilled pupa without stimulation did not secrete the prothoracic gland stimulation factor at the time of the isolation, while someone with stimulation secreted that factor at the same time. The results of the transplantation experiments in non-chilled pupae indicate that the electrical stimulation of the pupa accerelated the activation of the secretory function of the brain.

Table 6 spontaneous electrical activity of donor pupae and the development of donor and recipient pupae

pupa	electrical activity (peaks/10 sec.)		development	
	3-day	5-day	donor	recipient
A 1	18.5±3.26	17.3±1.91	−	+++
A 2	2.4±1.20	8.7±1.49	−	−
A 3	5.4±2.69	6.1±1.40	−	−
A 4	6.7±1.39	10.9±2.68	−	+
A 5	4.3±0.75	17.5±1.73	−	+
A 6	6.6±5.15	21.8±1.57	−	+
A 7	3.4±0.49	3.2±0.69	−	−
C 1	5.0±1.15	4.5±0.67	−	−
C 2	4.3±1.14	4.5±0.78	−	−
C 3	7.3±1.41	10.5±1.82	++	++
C 4	23.6±2.15	6.4±1.50	++	−
C 5	18.5±1.50	25.0±2.10	++	+++
C 6	7.8±2.14	17.5±2.18	++	+++
C 7	16.7±1.25	15.0±4.08	++	+++
E 5	3.1±0.44	7.0±1.26	−	+
E 6	2.4±0.68	8.3±1.70	−	++
E 7	2.3±1.10	4.8±1.92	−	−

stability of the equipment : 1.6 ± 0.49 (peaks/10 sec.)

4. *The relation between the spontaneous electrical activity of the pupa and the accerelatory effect of its brain on the development.*

Table 6 indicated the spontaneous electrical activity of the pupa, which was recorded at 3 day of incubation, just after the finishing of the stimulation, and 5 day of incubation through the pair of implanted electrodes. The value of the activity of the pupa was represented with the mean value of the numbers of the electrical fluctuation for random 10 seconds. Of these spontaneous electrical activities of the pupae, the values of E_5, E_6 and E_7 were those recorded from the non-stimulated pupae. In addition to the electrical activities, Table 6 indicated the development of the decerebrated pupa and that of the recipient non-chilled pupa. The value of the spontaneous electrical activity of the pupa was largely different with individual pupa. Particularly, the value of the stimulated pupa largely differed with individual.

The relation between the spontaneous electrical activity and the development of the pupa extirpated of the brain at 5 day of incubation was present in Fig. 2, Fig. 3 and Table 7. In these figures the value of the spontaneous electrical activity represented the value as that in Table 6, and the groups of pupae A, C and E represented the experiment (A), (C) and (E) respectively. Hollow circles represented the value in the pupa in which no development occurred, and solid circle represents that where development occurred when the pupa was extirpated

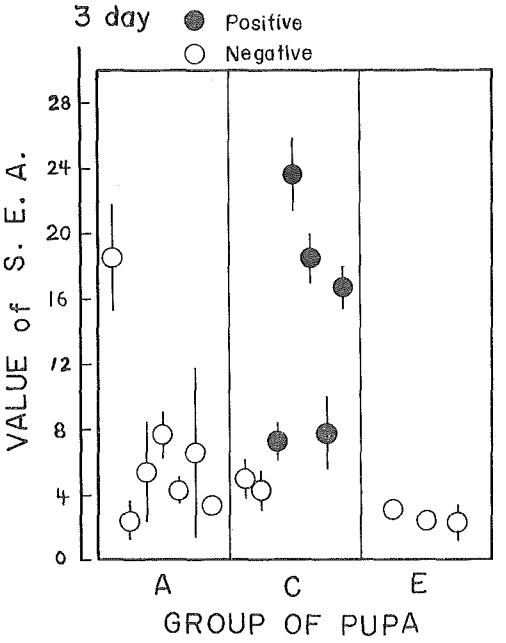

Fig. 2 relation between the spontaneous electrical activity at 3-day incubation and the development of the pupae extirpated of their brains at 5-day incubation

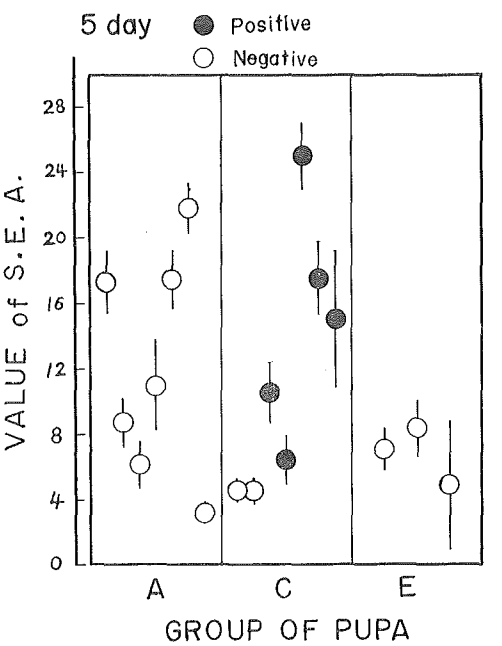

Fig. 3 relation between the spontaneous electrical activity at 5-day incubation and the development of the pupae extirpated of their brains at 5-day incubation

Table 7 relation between the spontaneous electrical activity of the pupae and the development of the pupae extirpated of their brains

develop.	expr.	No. of pupae	electrical activity (peaks/10 s.)	
			3-day	5-day
+	C	5	14.8±6.31	14.8±6.31
−	C	2	5.0, 4.3	4.5, 4.5
−	A	7	6.8±4.85	12.2±6.32
−	E	3	2.6±0.35	6.7±0.48

of its brain. In these figures and table, the higher values of the spontaneous electrical activity both in 3-day and 5-day were clearly shown in the developed pupae in the experiment (C). No one pupa developed in the experiment (A). In these pupae, the spontaneous electrical activity of the 5-day seems to be somewhat larger than that of 3-day pupae, but largely variable with individuals. The pupae in the experiment (E) did not develop, and the spontaneous electrical activity of these pupae was small in both 3-day and 5-day pupae.

The relation between the spontaneous electrical activity of the donor pupa at 3-day incubation and 5-day incubation and the development of the recipient non-

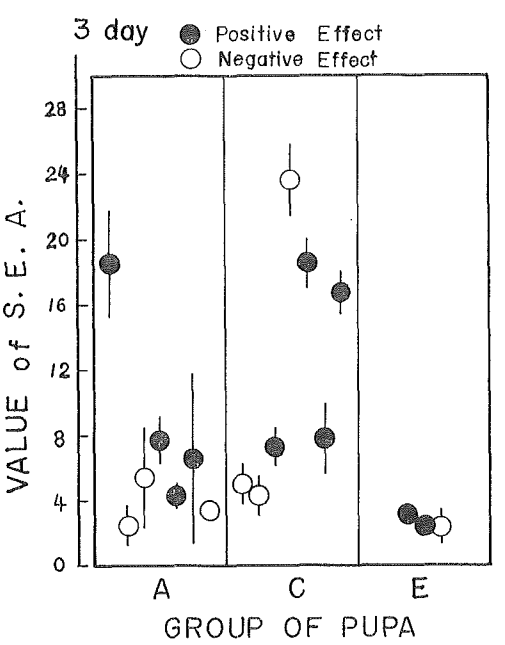

Fig. 4 relation between the spontaneous electrical activity of the donor pupae at 3-day incubation and the development of the recipient pupae transplanted the brains of donor pupae

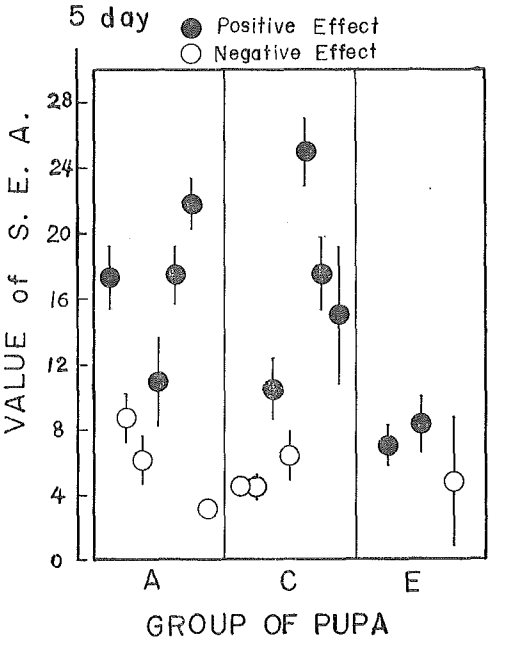

Fig. 5 relation between the spontaneous electrical activity of the donor pupae at 5-day incubation and the development of the recipient pupae transplanted the brains of donor pupae

Table 8 relation between the spontaneous electrical activity of the donor pupae and the development of the recipient pupae in the transplantation of the brain

develop. of recipient	donor pupae			
	expr.	No. of pupae	electrical activity (peaks/10 s.)	
			3-day	5-day
+	A	4	9.0±5.51	16.9±3.88
+	C	4	12.6±4.87	17.0±5.25
+	E	2	2.7±0.55	7.6±1.50
−	A	3	3.7±1.25	6.0±2.24
−	C	3	11.0±8.93	5.1±0.89
−	E	1	2.3±1.10	4.8±1.92
−	C*	2	5.0, 4.3	4.5, 4.5

* The value of the C 3 was excluded.

chilled pupa transplanted the brain isolated from the donor pupa at 5-day incubation was indicated in Fig. 4, Fig. 5 and Table 8. In these figures, hollow circle represents the value of the spontaneous electrical activity of the donor pupa when no development of the recipient non-chilled pupa occurred, and solid circle represents that of the donor pupa when the development of the recipient pupa occurred

in the transplantation experiments. A remarkable fact in these figures and table is that the non-chilled pupa developed when it was transplanted the brain of either the stimulated chilled or stimulated non-chiied pupa where somewhat larger spontaneous electrical activity was recorded at 5-day incubation. In contrast to this, the non-chilled pupa transplanted the brain of the pupa in which the smaller spontaneous electrical activity was recorded at 5-day incubation did not develop. In regard to the relation between the spontaneous electrical activity of the donor pupa at 3-day incubation and the development of the recipient pupa, no such clearly recognizable relationship as that at 5-day incubation seemed to be obtained. But when the results of the experiment (C) and (G) were considered except to a case in which the recipient pupa did not develop regardless the transplantation of the brain of the stimulated chilled pupa exhibiting high spontaneous electrical activity, the relationship resembled with that in 5-day activity seemed to be recognizable. In this case, the spontaneous electrical activity of the donor pupa at 5-day incubation was low. In the results of the experiment (A) and (B), the relation between the spontaneous electrical activity of the donor pupa at 3-day incubation and the development of the recipient pupa was not evident. In the results of the experiment (E) and (H), no relationship was recognized.

Discussion

It has been clarified by C. M. WILLIAMS that the initiation and maintenance of the pupal diapause of cecropia silkworm, *Hyalophora cecropia* (*Platysamia cecropia*), derived from the inactivation of the secretory function of the prothoracic glands of the insect, and the termination of the diapause occurred with the activation of the secretion of that gland. He found further that the inactivation and the activation of the prothoracic glands were induced by the inactivation and the activation of the secretory function of the neurosecretory cells of the brain, supraoesophageal ganglia, respectively. ('46b, '47, '52) VAN DER KLOOT discovered that the spontaneous electrical activity of the brain of the cecropia pupa remained inactive during diapause, while it became active 2 days before the least characteristics of the development of the pupa became apparent. In those cases, the abdominal nerve cord of the insect remained active regardless of diapause of the insect. ('55) In another giant silkmoth, *Samia cynthia pryeri*, which diapaused in the pupal stage, S. FUKUDA found that the diapause and the termination of diapause in the pupa were under control of the same mechanism as that in cecropia pupa. ('59) There is a report that the spontaneous electrical activity of the diapausing pupa of this insect is in an inactive state, and that of the developping pupa becomes in an active state. From the results of the extirpation of the brain of the pupa and the tranplantation of it, it has been sugested that the

inactive brain for the secretory function was inactive in electrical function on the one hand, and the active brain for the secretory function was active in electrical function on the other. (KOENUMA, A. '68)

It has been well known that the long exposure of the pupa to the low temperatures before the incubation is required for the further development of the pupa. VAN DER KLOOT reported that acetylcholine-choline esterase activity decreased in the non-chilled diapausing cecropia pupa, acetylcholine of the brain increased in the chilled pupa, and choline esterase activity of the brain increased correspond to the occurrence of the spontaneous electrical activity of the brain in the incubating chilled pupa. ('55) These facts suggest the possibility of the following events concerning to the termination of the diapause. Some nerve function is activated first by the thermal stimuli of the incubation in the brain of the chilled pupa, then the neurosecretory cells of the brain of the pupa begins to secrete the brain hormone respond to the any threshold stimuli of the nerve impulses, finally the activation of the prothoracic glands occurs in response to this hormone, thus the development of the pupa initiates in response to the increase of the prothoracic gland hormone. The indication of VAN DER KLOOT's experiments that the activation of the spontaneous electrical activity of the pupa occurred before the earlist characteristics of the initiation of the development of the pupa seems to suggest that the continuous action of the nerve for a definite period was required for the activation of the secretory cells of the brain. If such mechanism were required for the activation of the secretory cells of the brain, an earlier initiation of the secretion of the brain hormone will occur in the stimulated chilled pupa by the weak repetitive electrical pulses near the head region than in the non-stimulated one. Thus, development of the stimulated pupa will occur in spite of the extirpation of the brain at such period of incubation as no development of the non-stimulated pupa will be expected when the brain is removed. The possibility that the prothoracic gland of the pupa is activated by the direct action of the eletrical stimulation will be excluded when no development occurs in the stimulated pupa whose brain was removed before the stimulation.

Of the results in this investigation, it was only in the electrically stimulated pupa in which the result differed from the expectation with the supposition that the electrical stimulation had no effect on the activation of the brain secretion of the pupa. Any characteristics of the termination of the diapause did not occur in the pupa whose brain was removed before the stimulation. All unexpected results were those indicated the termination of the diapause. Therefore, it may be valid to consider that the brain of the pupa was activated by the repetitive weak electrical stimulation of the head region of the pupa. Particularly, the activation of the brain of the diapausing pupa by the electrical stimulation is

clearly indicated in the fact that the development occurred in the recipient non-chilled pupa which was transplanted the brain isolated from the stimulated non-chilled pupa. These results seem to confirm the VAN DER KLOOT's view that the reduction of the secretion of the brain was derived from the cessation of a nerve function and the initiation of the secretion of the brain was derived from the activation of the nerve function of the brain which was represented in the spontaneous electrical activity.

We must take into consideration the fact that the electrical signal of the pupa recorded through the pair of the implanted electrodes at the head region and the dorsal region of the 4-th abdominal segment will be composed of the spontaneous electrical activity of the brain, action potential of heart of the pupa, spontaneous electrical activity of suboesophageal ganglion and abdominal nerve cord, muscular action potential and potential change with respiratory movement of the pupa. In these experiments, the mean value of the numbers of the eletrical fluctuation for random 10 seconds was employed as the indication of the spontaneous electrical activity of the pupa. When such value was used, the potential change with the respiratory movement of the pupa may be neglected because of its negligible frequency for 10 seconds. The effect of the potential change derived from the heart movement on the value of the spontaneous electrical activity in these experiments seems to be negligible, for no difference between the pupa with brain and the pupa without brain is expected to develop because of the occurrence of the common frequency of the potential change, two or three times per 10 seconds, with the pulsation of heart in both pupae regardless the existence of their brains. The effect of the action potential by the muscular movement of the pupa on the value of the electrical activity in these experiments may be negligible for the reason that almost no movement of the pupa was recognized during the recording time and for the reason that no difference of the movement was indicated regardless the stimulation of the pupa. In these experiments, no spontaneous electrical activity of the abdominal nerve cord seems to be recorded in spite of the VAN DER KLOOT's report that the abdominal nerve cord remained active in both the diapausing and developping pupae. From the consideration above mentioned, it seems to be reasonable that the difference in the values in these experiments was regarded as the representation of the difference in the spontaneous electrical activity of the pupal brain. According to KOENUMA ('68), the chilled pupa extirpated of the brain at 5-day incubation did not develop and indicated the fact that the brain still remained inactive for the secretory function at that time of incubation. In these experiments, no chilled pupa extirpated of the brain at 5-day incubation without electrical stimulation developed. This fact indicates that the no neurosecretory cells still initiated secretion,

and this is consistent with KOENUMA's previous observation.

That the half of the chilled pupae extirpated of their brains at 5-day incubation with electrical stimulation developed indicates that the shortening of the period requirring for the activation of the neurosecretory cells of the brain occurred in the electrically stimulated pupae. This suggests the acceleratory action of the electrical stimulation for the activation of the secretory function of the brain. No non-chilled pupa developed in the case in which the brain was removed at 5-day incubation. There were two kinds of pupae, with a high electrical activity value on the one and with a low value on the other at 5-day incubation. The non-chilled pupa transplanted the brain isolated from the former pupa developed, while the non-chilled pupa transplanted the brain isolated from the latter pupa did not develop. This seems to indicate that even in the non-chilled pupa the secretion of the brain occurred respond to the electrical stimulation, and that the brain thus activated presented the high spontaneous electrical activity. This fact does not contradict with the observations of VAN DER KLOOT ('55) and KOENUMA ('68) where the spontaneous electrical activity occurred before the secretion of the brain.

When the relation between the spontaneous electrical activity of the pupa and the development of the pupa extirpated of its brain at 5-day incubation was considered, following facts were found. Only in those pupae indicating high spontaneous electrical activity both in 3-day and 5-day incubation, development occurred. Contrast to this, development did not occur in those pupae indicating low electrical activity both in 3-day and 5-day incubation or either in 3-day or 5-day incubation. These facts suggest that a somewhat continuation of an electrically active state of the brain was required for the activation of its secretory function. Therefore, these facts seem to make more probable the possibility of the mechanism that some nerve impulses initiate first in the brain, next the activation of the secretory cells occurs in response to a difinite threshold of the nerve impulses.

When the relation between the spontaneous electrical activity of the donor pupa and the development of the recipient pupa transplanted the brain of the donor pupa was considered, following facts were found. Development of the recipient pupa occurred when the brain of the electrically stimulated pupa indicating high spontaneous electrical activity at 5-day incubation was transplanted, while no development of the recipient pupa occurred when the brain of the stimulated pupa indicated low electrical activity at 5-day incubation. These facts seem to indicate that the brain of the stimulated pupa was already electrically active at 5-day incubation and the high spontaneous electrical activity of the pupa may represent the high spontaneous electrical activity of the brain. That

no development occurred in the recipient pupa by the transplantation of the brain of the stimulated pupa with low electrical activity may be explained that in this case the brain of the donor remained inactive at 5-day incubation and it remained still inactive even after the transplantation by some unknown causes. No relationship found between the spontaneous electrical activity of the non-stimulated doner pupa and the development of the recipient pupa transplanted the brain of the donor. This fact seems to indicate that the brain of the non-stimulated chilled pupa remained inactive in 5-day incubation. Therefore, this fact is not contradict with the previous report of KOENUMA ('68). In this case, the activation of the brain seems to occur after the transplantation.

No special relationship seemed to be found between the spontaneous electrical activity of the donor pupa at 3-day incubation and the development of the recipient pupa transplanted the brain of the donor which was isolated at 5-day incubation.

From the present discussion on the relation between the spontaneous electrical activity of the donor pupa and the effect of the transplantation of the brain of the donor on the development of the recipient pupa, it seems appropriate to conclude that the termination of the diapause was promoted by the electrical stimulation of the pupa and that the spontaneous electrical activity of the brain occurred before the activation of the secretory function of the brain.

The results of present experiments seem to increase the possibility that the activity of the secretory cells of the pupal brain which is concerned in the diapause of the pupa of this insect is controled by the nerve impulses.

The author wishes to express his thanks to Dr. S. FUKUDA for his direction and encouragement in this study. He also wishes to express his gratitude to Professor H. KURASAWA of the Shinshu University for his criticism and invaluable help in preparing the manuscript.

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