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The Electrical Activity of the Pupal Brain in the Diapausing and Developing Pupa of the Giant Silkmoth, Philosamia cynthia cynthia.

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Abstract

The electrical activity of the pupal brain of the giant silkmoth, *Philosamia c. cynthia* was measured during diapausing and developing period of the pupa. The endocrinological activity of the brain was tested by means of the extirpation and the transplantation of the brain after the measurement of the electrical activity.

The electrical activity of the pupal brain was inactive in the diapausing pupae. It became active in some marginal periods of the incubation at higher temperatures after chilling. Results of the extirpation and transplantation experiments of the brain clearly showed that the electrically inactive brain of the pupa was also in the endocrinologically inactive state, and the electrical activity of the pupal brain occurred previous to the endocrinological activity. These results obtained from the present experiments were in accordance with the WILLIAMS' results and the fact suggested by VAN DER KLOOT.

Introduction

The role of the brain upon the beginning and ending of the pupal diapause of the giant silk worm, *Hyalophora cecropia*, has been clairfled by C. M. WILLIAMS. According to W. G. VAN DER KLOOT,[3] in this pupa the brain was electrically silent during diapause, and then it became electrically active two days before the first appearance of the adult development occurred. He concluded that the change in the electrical activity of the pupal brain was connected with the diapause of the pupa though he did not show the direct relation between the electical activity and the endocrinological activity of the brain.

The ending of the pupal diapause in cecropia silk worm occurs when the diapausing pupa is incubated at higher temperatures after the relatively long exposure to the lower temperatures. It has been reported that there was a marginal period of the incubation for the functional change of the brain (WILLIAMS[5]).

No adult development of the pupa resulted from the removal of the brain before the marginal period on the one hand, development to the moth resulted from the removal of the brain after the marginal period on the other. The implantation of the previously chilled brain obtained from the pupa before the marginal period produced the ending of the diapause of the recipient pupa, but the implantation of the brain obtained after the marginal period produced the no adult development of the recipient pupa (WILLIAMS[5]).

According to FUKUDA, S.,[1] the role of the brain on the pupal diapause in the other giant silkworm, *Philosamia cynthia cynthia*, resembles that of the cecropia silkworm.

In present paper, there is a report of the experiments carried out to investigate the relation between the electrical activity of the brain and the endocrinological activity of the brain of the pupa of *Philosamia cynthia cynthia* during the developmental process.

Material and Methods

The first group of the pupae of *Ph. c. cynthia* used for experiments were collected near Matsumoto in October. They were stored intact in an incubator at 26°C till the next June. 9 out of 69 pupae developed to the imago during the storage period, 25 pupae survived in diapausing state, and the rest pupae died. 10 pupae out of these surviving pupae were chilled for 8 weeks at 5°C with 78% humidity, then they were used for experiments.

The second group of the pupae were collected in September. They were stored at 25°C with cocoon and cocoons were removed just before the use. In this group, non out of 22 pupae developed to the imago, 6 survived in diapausing state, and 16 died during the storage at 26°C until the late in next May. This 6 surviving pupae were chilled for over 8 weeks and were used for experiments.

The third group of the pupae collected late in October were stored with cocoon at 26°C. Some of these pupae were chilled at 6°C from December to early May and used for experiments. In this group, non out of 68 pupae developed to the imago during storage at 26°C, 25 pupae survived in diapausing state, and 43 pupae died. The any significant difference of the experimental result was not found among three groups of the pupae.

The electrical activity of the pupal brain and other nervous tissues of the pupa which was incubated at 26°C for a given period after the chilling was measured by means of the Ag-AgCl electrodes. After the measurement of the electrical activity, the brain was removed from the pupa, and in some cases the isolated brain was transplanted to the diapausing pupa. The development of the decerebrated pupa and the development of the recipient pupa were observed at

26°C. All treatments on the pupa was carried out through a small window of the integument at head region. This integumental window was sealed by melting paraffin after the treatments were finished.

To protect the pupa from infectious organsms, the treatment was carried out in a small room sterilized by a ultra-violet light irradiation, by use of sterilized instruments, and before paraffin sealing penicillin and streptomycin powder were added. To protect from the toxic darkening change of the pupa, some peletts of phenyl thiourea crystals were also sealed. When the pupa was treated, it was narcotized by Williams' carbon dioxide gas method.[4]

In the electrical measurement of the pupa, the following instruments were used. A Nippon-Koden VC-7 oscilloscope with an AVH-2 preamplifier and a WC-10B recording camera. Recording electrodes were Ag-AgCl electrodes tapered electrolytically and insulated with glass capillary tubes except their tips. Indifferent electrodes were Ag-AgCl wires. To stimulate the pupa a Nippon-Koden MSE-3 electronic Stimulator with an isolating unit was used. To insert the electrode to the tissue a Peterfi type micromanipulator was used. Measurements were carried out at 20 to 22°C.

Results

(1) *Electrical activity of the pupal brain during incubation at 26°C after chilling.*

Table 1 indicates the results of the measurement of the electrical activity of the pupal brain and other tissues during incubation at 26°C after chilling. This table also indicates the ability to respond of the brain to the stimulation. Showing clearly in this table, the pupal brain incubated not exceeding 6 days showed neither a spontaneous electrical activity nor a response to the stimulus. Contrasting to the pupal brain, the suboesophageal ganglia showed the electrical activity throughout the incubation. The pupal brain incubated for over 6 days became electrically active and to respond to the stimulus in some cases. A definit relation seems to exist between the spontaneous electrical activity of the pupal brain and its respondability to the stimulus. That is on the one hand the brain shows the electrical activity can respond to the stimulus, on the other the brain silent in electrical activity can not respond to the stimulus. Fig. 1, 2 and 3 showed the electrical activities of the typical electrically inactive brain, active brain and the electrically active suboesophageal ganglion respectively.

(2) *Development of the chilled pupae removed their brain after the definite periods of incubation.*

Table 2 indicates the results of the removal of the brain from the pupae which were incubated for the definite periods after the chilling. In this table, it was clearly shown that the pupae removed the brains within 5 days of incuba-

Table 1 Electrical activity of pupal brain and some nervous tissues in the pupae of *Ph. c. cynthia*

Pupa	incubation period	electrical activity		excitability of brain
		brain	suboesophageal ganglion	
3131	0day	−	+	−
1315C	0	−	+	−
3112A	5	−	+	−
3115A	5	−	+	−
7281	5	−	+	−
1	6	−	+	−
2	6	−	+	−
3	6	−	+	−
3174	6	+	+	+
3175	6	−	+	−
3176	6	+	+	+
3195	7	+	+	+
7301	7	−	+	−
4	8	+	+	+
5	8	+	+	+
6	8	−	+	−
3141A	8	−	+	−
3142A	8	+	+	+
3143A	8	+	+	+
3205	8	+	+	+
3206	8	+	+	+
3207	8	+	+	+
7311	8	+	+	+
3151A	9	+	+	+
3152A	9	−	+	−
3153A	9	+	+	+
8011	9	+	+	+
3161A	10	+	+	+
7	11	+	+	+
8	11	+	+	+
9	11	+	+	+
3235	11	+	+	+
3236	11	+	+	+
3181A	12	+	+	+
3182A	12	+	+	+
3183A	12	+	+	+
3245	12	+	+	+
8071	15	−	+	−
8072	15	+	+	+

+ : active, − : inactive

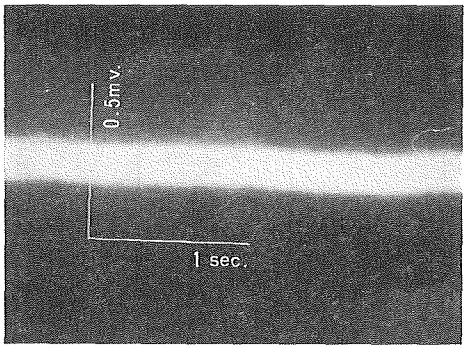

Fig. 1 electrical activity of an inactive brain, 5 days after incubation at 26°C with previous chilling at 6°C.

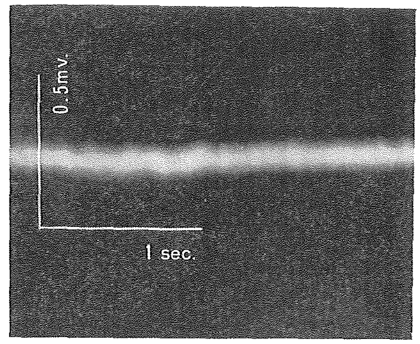

Fig. 2 electrical activity of an activated brain, 8 days after incubation at 26°C with previous chilling at 6°C.

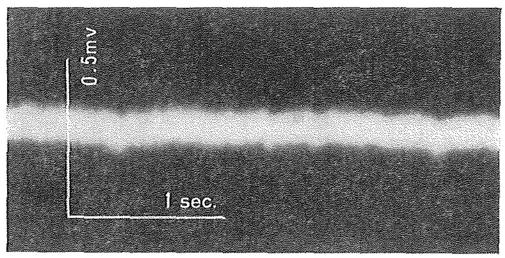

Fig. 3 electrical activity of a suboesophageal ganglion, in a chilling pupa at 6°C.

tion did not develop to the imago but continued the diapause. Table 2 indicates also that when the extirpation of the brain was carried out in the pupae incubated longer than 6 days, some of the pupae initiated the adult development.

Showing in Fig. 4, the pupa remaining the brain developed to imago after incubation.

(3) *Development of the diapausing pupa implanted the brain obtained from the pupa incubated for a definite period after the chilling.*

The effect of the transplantation of the brain obtained from the pupa incubated for a definite period with previous chilling to the diapausing pupa is present in Table 3. Showing in this table, the recipient diapausing pupa implanted the brain obtained from the pupa incubated shorter than 5 days initiated the adult development. The results, however, varied according to each case when transplanted brains were obtained from the pupae incubated for a longer period than 7 days.

Fig. 5 indicates a typical case, where the pupa removed the brain at 5-th day of incubation (A) failed to adult development (B), the pupa implanted the brain from the above mentioned pupa (C) formed the adult moth (D).

Table 2 Effect of the extirpation of the brain from the incubating chilled pupa on the development. *Philosamia cynthia cynthia*

pupa	period of extirpation	survival period of pupa	development	effect of extirpation	remarks
1315C	0 days of incubation	45 days	−	+	
3131	0	45	−	+	
3112A	5	28	+	−	Extirpation. was incomplete.
3115A	5	28	−	+	
7281	5	60	−	+	
1	6	90	−	+	
2	6	90	−	+	
3	6	66	−	+	
3174	6	34	++	−	
3175	6	45	−	+	
3176	6	45	++	−	
3195	7	45	++	−	
7301	7	60	−	+	
4	8	66	+	−	
5	8	41	−	+	
6	8	80	−	+	
3141A	8	30	−	+	
3142A	8	42	++	−	
3143A	8	42	−	+	
3205	8	45	++	−	
3206	8	45	++	−	
3207	8	45	++	−	
7311	8	75	++	−	
3151A	9	41	++	−	
3152A	9	70	−	+	
3153A	9	60	+	−	
8011	9	60	−	+	
3161A	10	124	+	−	
7	11	70	++	−	
8	11	56	++	−	
9	11	56	++	−	
3235	11	45	++	−	
3236	11	45	++	−	
3181A	12	122	++	−	
3182A	12	122	−	+	
3183A	12	122	+	−	
3245	12	45	++	−	
8071	15	67	−	+	
8072	15	77	++	−	
10	−	58	+++		
3184A	−	45	+++		

incubation : 26°C
chilling : 6°C for 8 weeks
development;

− : no sign of adult development
+ : sign of adult development —— separation of integument molting fluid between skin and integument.
++ : Adult skin is formed completly.
+++ : Moth is formed completly.

effect of removal ; + : effective, − : ineffective

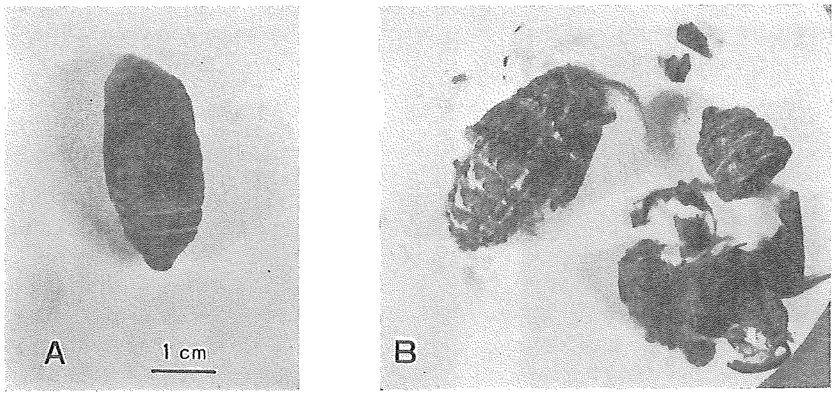

Fig. 4 Development of a pupa remained the brain.
A : diapausing pupa
B : after adult development

Table 3 The effect of the brain of the chilled pupa on the diapausing pupa of *Ph. c. cynthia*

recipient pupa	donor pupa	period of implantation	development of recipient	survival period of rupa	remarks
3112 B	3112 A	5 days of incubation	++	23 day	
3115 B	3115 A	〃	+++	60	Imagination
11	1	6	++	84	
12	2	〃	+++	56	Imagination
13	3	〃	++	80	
14	4	8	—	84	
16	6	〃	+	80	
3141 B	3141 A	〃	++	30	
3142 B	3142 A	〃	—	128	
3143 B	3143 A	〃	+++	45	Imagination
3151 B	3151 A	9	—	60	
3152 B	3152 A	〃	+++	54	Imagination
3153 B	3153 A	〃	—	124	
3161 B	3161 A	10	—	60	
17	7	11	—	70	
18	8	〃	—	56	
19	9	〃	—	56	
3181 B	3181 A	12	—	123	
3182 B	3182 A	〃	+++	60	Imagination
3183 B	3183 A	〃	—	134	

development ; — : no initiation of adult development
+ : occurance of the initiation of adult development
++ : formation of the adult skin
+++ : formation of complete moth

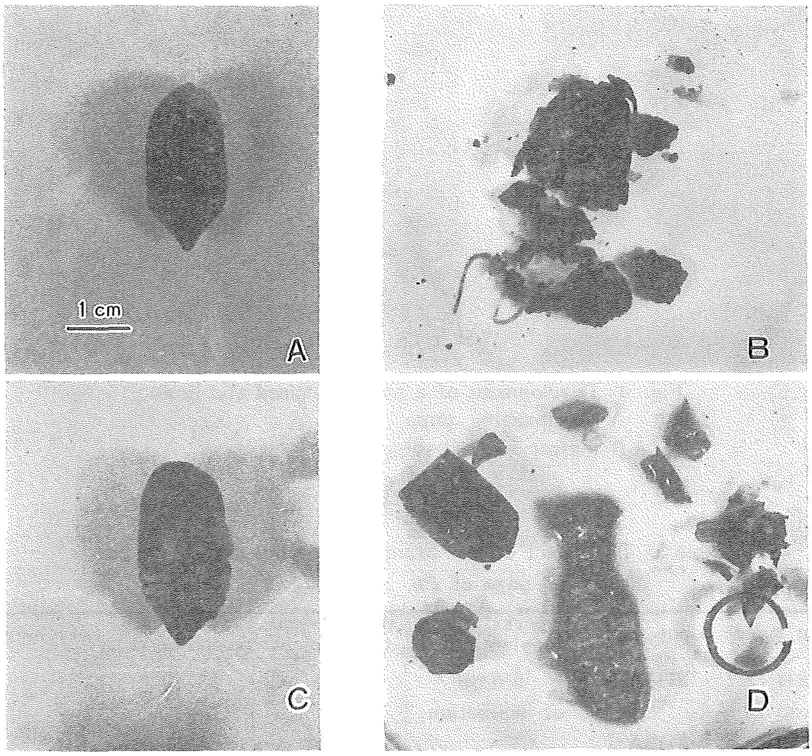

Fig. 5 a typical case where no initiation of the adult development of the pupa resulted from the extirpation of the electrically inactive brain and the formation of the moth resulted from the transplantation of the above mentioned brain to the diapausing pupa.
A and B : the development of the decerebrated pupa
C and D : the development of the transplanted pupa

(4) *A relation between the electrical activity of the pupal brain and the effect of the extirpation or the transplantation of the brain to the diapausing pupa on the development.*

Table 4 shows the result of the measurement of the electrical activity of the pupal brain and the result of the extirpation of the brain or the result of the transplantation of the brain to the diapausing pupa in one table. Clearly in the table, no initiation of the adult development of the pupa resulted from the extirpation of the still electrically inactive brain. While the initiation of the adult development resulted from the extirpation of electrically active brain in some pupae.

The adult development of the recipient pupa resulted from the transplantation of the still electrically inactive brain to the diapausing pupa. Contrasting with the transplantation of the electrically inactive brain, the trnsplantation of the electrically active brain produced no initiation of the adult development of the pupa in one case, or produced the initiation of the adult development of the

Teble 4 Relation between the electrical activity of the pupal brain and the effect of the extipation or implantation of the brain on the development of the pupa of *Ph. c. cynthia*

donor pupa	period of extirpation of brain	electrical activity of brain	development of decerebrated pupa	development of implanted pupa	recipient pupa
1315 C	0 day incubation	−	−		
3131	0	−	−		
3175	5	−	−		
7281	5	−	−		
7301	7	−	−		
8071	15	−	−		
3112 A*	5	−	+	++	3112 B
3115 A	5	−	−	+++	3115 B
1	6	−	−	++	11
2	6	−	−	+++	12
3	6	−	−	++	13
3141 A	8	−	−	++	3141 B
6	8	−	−	+	16
3152 A	9	−	−	+++	3152 B
3174	6	+	++		
3176	6	+	++		
3195	7	+	++		
3205	8	+	++		
3206	8	+	++		
3207	8	+	++		
5	8	+	−		
7311	8	+	++		
8011	9	+	−		
3235	11	+	++		
3236	11	+	++		
8072	15	+	++		
4	8	+	+	−	14
3142 A	8	+	++	−	3142 B
3143 A	8	+	−	+++	3143 B
3151 A	9	+	++	−	3151 B
3153 A	9	+	+	−	3153 B
3161 A	10	+	++	−	3161 B
7	11	+	++	−	17
8	11	+	++	−	18
9	11	+	++	−	19
3181 A	12	+	++	−	3181 B
3182 A	12	+	−	+++	3182 B
3183 A	12	+	+	−	3183 B

* The removal of the brain was incomplete.
electrical activity ; + : active, − : inactive.
development ; − : no initiation of adult development.
+ : occurance of the initiation of the adult development.
++ : formetion of adult skin.
+++ : formation of complete moth.

pupa in the other case.

On the relation between the effect of the extirpatiom of the brain and the transplantation of the removed brain, an apparent relation was observed. The transplantation of such a brain as the failure of the initiation of the adult devel-

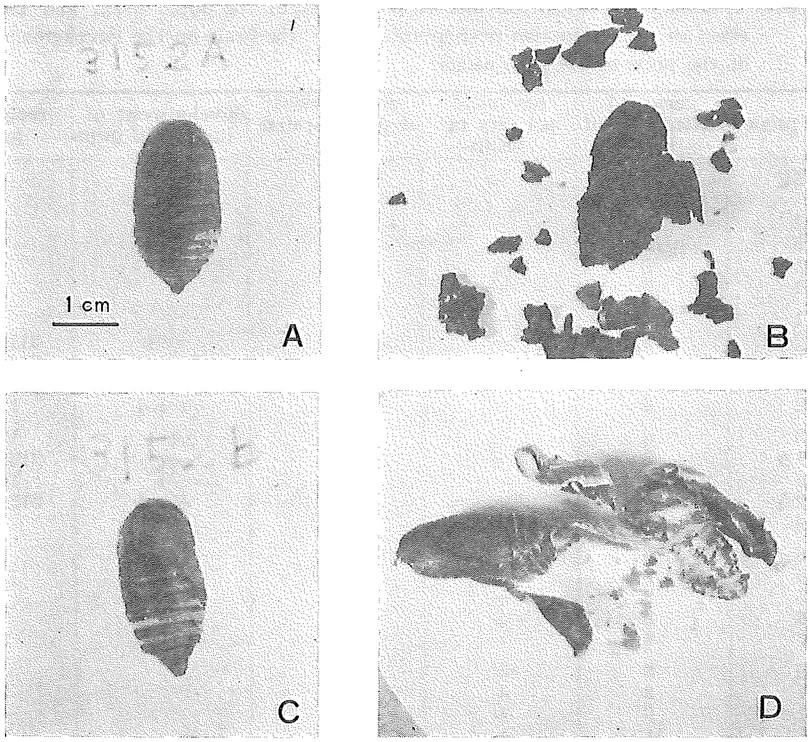

Fig. 6 a typical case where no initiation of the adult development resulted from the extirpation of the electrically active brain of the pupa and the formation of the moth of the diapausing pupa resulted from the transplantation of the above mentioned brain to the diapausing pupa.
A and B : the development of the decerebrated pupa.
C and D : the development of the trans planted pupa.

opment of the donor pupa resulted from the extirpation of the brain always produced the initiation of the adult development of the recipient pupa, and the transplantation of such a brain as the initiation of the adult development of the donor pupa occurred in spite of the extirpation of the brain always produced the failure of the initiation of the adult development of the recipient pupa.

Fig. 6 shows such a case as the removal of the electrically active brain produced the failure of the initiation of the adult development of the pupa on the one hand (A and B), and the implantation of the brain obtained from the above mentioned pupa produced the initiation of the adult development of the recipient pupa on the other hand (C and D).

In the pupa of 3112A, there was an exceptional case, where the decerebrated pupa developed considerablly in spite of the extirpation of the electrically inactive brain. In this case the extirpation of the brain was incomplete, so that the initiation of the adult development of the pupa seems to result from the fragment of the brain still remained in the pupa.

Discussion

The mechanism of the role of the brain upon the beginning and ending of the pupal diapause of the giant silk worm, *Hyalophora cecropia* (Lepidoptera), has been clarified by C. M. WILLIAMS.[5~7] In the pupal diapause of the other giant silk moth, *Philosamia cynthia cynthia*, it has been clarified by S. FUKUDA[1] that the pupal diapause of the insect was controlled by the brain through the similar mechanism as that of the cecropia silkworm. In these insects, to activate the pupal diapause, it is required that the insects were exposed to low temperatures for considerablly long period previously to the incubation at higher temperatures. When the previously chilled cecropia pupa was incubated, it was reported by WILLIAMS[5] that there was a marginal period on an activation of the brain. According to him, this marginal period was different with each individual pupa, and it was distributed between 11 and 17-day incubation at 25°C. W. G. VAN DER KLOOT[3] reported that the cecropia brain was electrically inactive during diapause and it became electrically active two days before the first appearance of the adult development occurred. In the case of VAN DER KLOOT, the initiation of the adult development occurred between 7 and 16 days of incubation at 25°C in the previously chilled pupae. Contrasting with the brain, he reported that the suboesophageal ganglion or ventral nerve cord was electrically active during diapause. In the present experiments, the brains of the previously chilled cynthia pupae during the incubation at 26°C were all electrically inactive before 5 days of the incubation, and became electrically active after 6 days of incubation in some pupae as presenting in table 1. The electrical activity of the suboesophageal ganglion was always active. This result was consist with that of VAN DER KLOOT.

TYSHCHENKO, V. P.[2] reported that the inhibition of the spontaneous bioelectric activity of the nervous system of the Lepidopteran pupae was not connected with the temporary termination of the neurosecretory cells activity of the diapausing pupa. In this regard the result of the present experiment was not according to TYSHCHENKO'S.

When the pupa incubating at 26°C with previous chilling was decerebrated before 5 days of incubation, no initiation of the adult development occurred. When the extirpation of the pupal brain was carried out after 6 days of incubation, the effect of the decerebration on the initiation of the adult development of the pupa varied according with each case. In some cases where the pupa decerebrated after 6 days of incubation, the initiation of the adult development occurred in spite of the decerebration, in other cases, however, no initiation of the adult development occurred in the decerebrated pupa. According to WILLIAMS[5] the marginal periods, when the decerebration in the incubating pupae of cecropia silk worm chilled previously initiate no adult development, varied according with each pupa, and they were distributed between 11 days and 17 days of incubation at 25°C. According to VAN DER KLOOT[3] the cecropia pupae chilled previously at 6°C for 13 weeks initiated the adult development after 6 to 16 days of incubation at 25°C. In the present experiments, the fact that the effect of the decerebration of the pupae on the development of the pupae of *Ph. c. cynthia* differed according with the time when the decerebration was carried out, may be explained by the marginal period for the nourosecretory activation of the pupal brain. The fact that the various results were produced when the pupae were decerebrated after longer than 6 days incubation at 26°C may also be explained by the variation of the marginal period for the activation of the pupal brain. In this regard, the present result of *Ph. c. cynthia* resembled to that of cecropia silkworm by WILLIAMS.[5]

According to WILLIAMS,[5] in cecropia silkworm the diapausing pupa implanted the brain obtained from the incubating pupa within the marginal period initiates the adult development, but the diapausing pupa implanted the brain obtained from the incubating pupa after the marginal period does not initiate the adult development.

In the present experiments, showing in the table 4, the adult development of the pupa was initiated in the diapausing pupa implanted the brain obtained from the pupa of which adult development failed to initiate by the decerebration, and the adult development of the diapausing pupa failed to initiate in the pupa implanted the brain obtained from the pupa of which adult development was initiated in spite of the removal of the brain. In the present results of the implantation of the brain in cynthia pupae consisted with the WILLIAMS' results in cecropia silkworm.

On the electrical activity of the pupal brain, VAN DER KLOOT[3] reported that the pupal brain was electrically inactive in diapausing and it became electrically active previous to the initiation of the adult development in the cecropia silkworm. He suggested that the electrical activity of the brain occurred before the secretion of the brain hormone, but he did not show the direct evidence of the the relation between the electrical activity and the secretion of the hormone of the pupal brain. In the present experiments the adult development was not initiated in the diapausing cynthia pupa when the electrically inactive brain was removed. Studying by WILLIAMS[5] and by FUKUDA,[1] when the pupal brain in these pupae was removed previous to the marginal period for its humoral activation, no adult development of the decerbrated pupa was initiated. It is quite probable, therefore, that the electrically inactive brain of the diapausing pupa is inactive in this insect. When the electrically active brain was removed from the pupa, two quite different results were produced according with each case. In one case the adult development of the pupa initiated in spite of the extirpation of the brain, and in the other no adult development of the pupa resulted from the extirpation of the brain. In the case where the pupal brain was electrically active without the initiation of the adult development in the extirpation of it, it is reasonable to consider that the pupal brain remained still inactive, for the transplantation of such a brain to the diapausing pupa was followed by the initiation of the adult development. In the other case where the pupal brain was electrically active with the initiation of the adult development of the decerebrated pupa, it is reasonable to consider that the brain hormone was already secreted, for the transplantation of such a brain to the diapausing pupa was followed by no initiation of the adult development of the pupa. The results obtained in the present experiments, therefore, seem to accord with the VAN DER KLOOT's results.[3]

In the present experiments, the electrical activity in the nervous tissue other than brain was active regardless of the state of the pupa. This result is also consistent with one of the VAN DER KLOOT's results.[3]

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