Follicular Degeneration in Unfed Nulliparous Females of *Culex tritaeniorhynchus*

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Abstract: With unfed nulliparous females of *Culex tritaeniorhynchus*, the most important vector of Japanese encephalitis, the follicular degeneration was examined under various conditions. Most of first follicles in an ovary were found to degenerate commonly in gonoactive females which emerged in early September and were kept at outdoors for a long period of about 3 months without being allowed to take blood meal. However, in gonoinactive, or diapausing, females having emerged after middle September when the day-length becomes shorter and the air temperature falls, degenerate follicles were low in rate, even when they were held for about 3 months or more without blood meal. Unfed nulliparous females with many degenerate follicles were scarcely found in autumn and early spring among those which were caught at animal sheds. Accordingly, it is considered that follicular degeneration in gonoactive females of the present species is commonly shown under experimental conditions, however, such a phenomenon rarely occurs in the field.

It is known that follicles commonly degenerate and show the similar morphology to dilatations which are formed in ovarioles after oviposition, when mosquito females feed on small amount of blood meal, or on animal blood which is nutritionally inadequate for follicular development (Clements, 1963 ; Detinova, 1962 ; Hosoi, 1954 ; Mogi et al., 1972). The follicular degeneration has been reported to occur also in unfed nulliparous females of the genus *Culex* (Bellamy and Corbet, 1974 ; Kupriyanova, 1967 ; Oda and Kuhlow, 1973 ; Spielman and Wong, 1973). We noticed the same phenomenon in unfed nulliparous females of *Culex tritaeniorhynchus*, which is the most important vector of Japanese encephalitis, when the physiology of diapause of the present mosquito was investigated. In the present paper, the factors to produce degenerate follicles in this mosquito are reported.

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MATERIAL AND METHODS

Abonrmal ovarioles (with the degenerate follicle) and normal ones (without the degenerate follicle) in an ovary were separately counted under a compound microscope with unfed nulliparous females of *Culex tritaeniorhynchus* (Nagasaki strain). The females examined were obtained (1) by rearing the lst instar larvae to adults under long photoperiod (16 hours) and high temperature (28°C), (2) by rearing the lst instar larvae until various develpmental stages (larval stages, pupal stage and newly emerged adult) under long photoperiod (16 hours) and high temperature (27°C), and then under short photoperiod (10 hours) and low temperature (21°C), and (3) by rearing the lst instar larvae to adults at outdoors from September to October.

Also, unfed females of *Culex tritaeniorhynchus* caught in pigsties in middle September were reared till December at an outdoor insectarium under natural conditions and examined for follicular degeneration. In addition, unfed females which had been caught in late March and early April were killed for examination just after collection. Such females were determined to be either nulliparous or parous by examining skein conditions of tracheoles attached to one ovary, and only with nulliparous females, abnormal ovarioles and normal ones in the other ovary were counted, because degenerate follicles in parous females were found by laboratory experiments to be often difficult to be distinguished from dilatations.

In these experiments, females were not generally allowed to take blood meal, but if necessary, the mosquitoes were permitted to feed on a chicken for one night. The developmental stages of follicles in this paper were as used by Kawai (1969).

RESULTS

1. *Morphology of ovarioles*

As ovarioles of various types were found in the present experiment, some examples are given in Photo. A to F.

Photo. A. A germarium and a first follicle in stage of N to Ib are contained in a normal ovariole without the degenerate follicle. The ovariole of this type usually appears in an unfed nulliparous femsle.

Photo. B. The ovariole has a germarium, a small follicle in early stage of No2, and a degenerate follicle. Such an ovariole is often observed in an unfed nulliparous and an unfed parous female as well as in a gravid female, although the ovariole of this type is very small in number in each ovary.

Photo. C. The ovariole consists of a germarium, a small-sized follicle in stage No2, and a degenerate follicle. The current follicle in stage No2 is more advanced in development than that shown in Photo. B. The ovariole given in Photo. C is commonly found in ovaries of gonoactive females which were kept for a long time without being allowed to take blood meal.

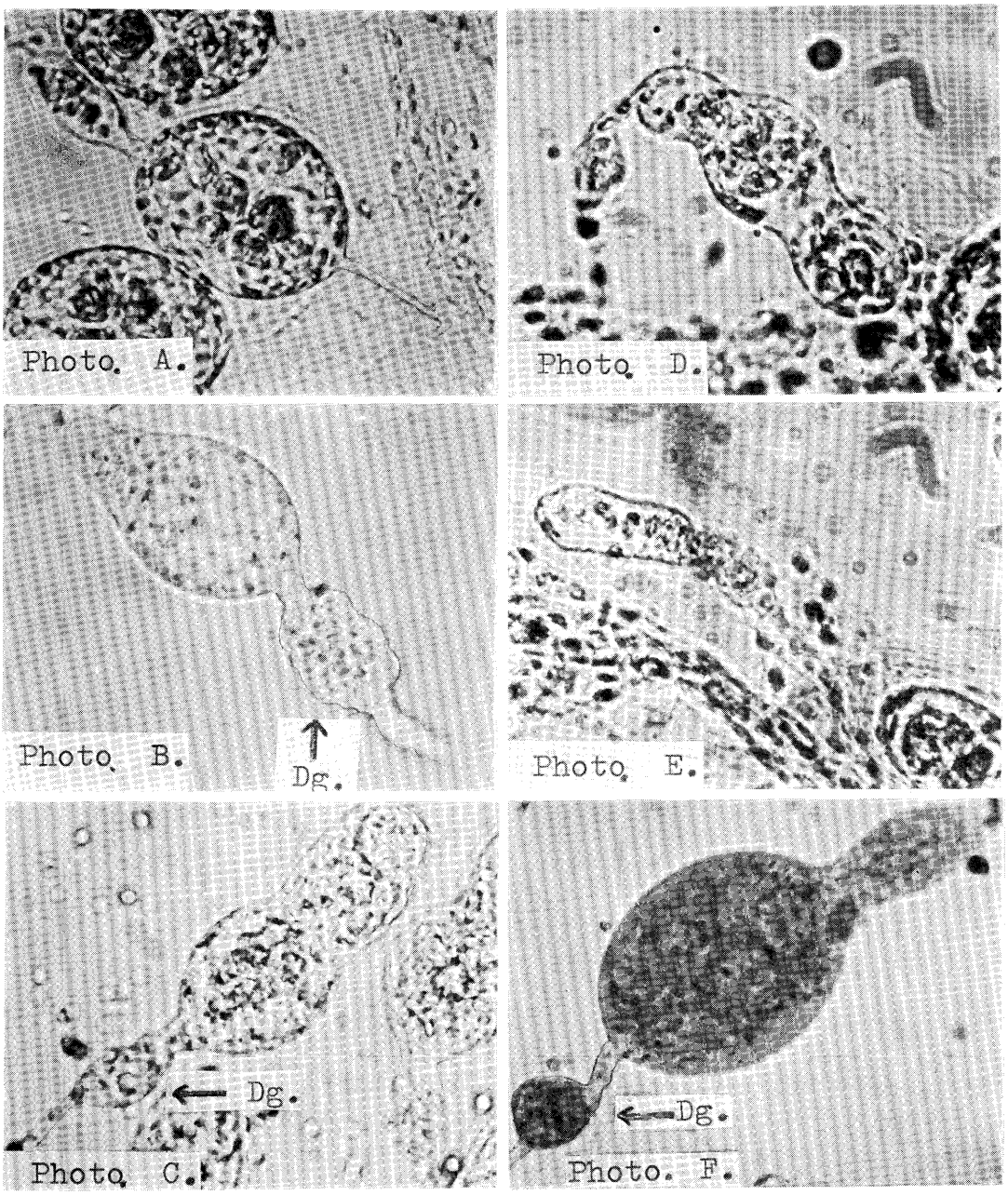

Photo. A. Ovariole with normal follicle.
Photo.B and C. Ovariole with degenerate follicle.
Photo. D and E. Ovariole which developed abnormally.
(The above ovarioles were obtained in unfed nulliparous females).
Photo. F. Ovariole with degenerate follicle formed after taking blood meal.
Dg. : Degenerate follicle.

Photo. D and E. These pictures show unusual ovarioles which have degenerate follicles and are rarely found.

Photo. F. The ovariole is composed of a germarium, a well-developed current follicle in the N stage, and a degenerate follicle, and is found usually in a female which takes a small amount of blood meal or on animal blood which is nutritionally inadequate for follicular development.

Since ovarioles were examined only with unfed nulliparous females, as written earlier, ovarioles as shown in Photo. F, found in fed females, are, of course, not included in the results of this experiment. The ovariole indicated in Photo. A is called the normal ovariole, and the others except for Photo. F. are called the abnormal ovariole.

2. Follicular degeneration in unfed females which emerged under long photoperiod and high temperature.

Degenerate follicles were counted with unfed females which emerged under experimental conditions with photoperiod of 16 hours and temperature of 28°C. The results are given in Table 1.

Table 1. Degeneration of first follicles in unfed females of *Culex tritaeniorhynchus* which were reared as adults from lst instar larvae under experimental conditions with 16 hour day-length and 28°C temperature

Days after emergence	No. dissected	No. females with degenerate follicles of following percentage of					
		0	-20	-40	-60	-80	-100
0	10	10					
3	10	3	7				
5	10	4	6				
7	10	4	6				
10	20	2	16	2			
25	8		2	2	2	0	2
40	10						10

From 3 to 7 days after emergence, abnormal ovarioles as shown in Photo. B appeared only in a low rate. Ten days after, abnormal ovarioles began to increase remarkably in number, and 40 days after, most of ovarioles had degenerate follicles. In females kept for 25 or 40 days, there occurred a great variation in the morphology of ovarioles, and most of them showed the morphology given in Photo. B to E.

3. Follicular degeneration in unfed females which were reared from the lst instar larvae until various developmental stages under 16 hour photoperiod and 27°C temperature, and thereafter under 10 hour photoperiod and 21°C temperature.

Degenerate follicles of abnormal ovarioles in an ovary were counted and the sizes of the first follicles of normal ovarioles in the same ovary were measured with unfed females which were reared from the lst instar larvae till various immature stages or adult emergence under

Table 2. Follicular degeneration and size of first follicles in unfed females of *Culex tritaeniorhynchus* which were transferred at each immature stage or just after emergence from condition with long photoperiod of 16 hours and 27°C temperature into condition of short photoperiod of 10 hours and 21°C temperature

Developmental stages* exposed to specific photoperiod and temperature		No. dissected**	No. females with degenerate follicles of following percentage of					No. females with first follicles of following size*** of									
16 hours, 27°C	10 hours, 21°C		0	-10	-20	-30	-40	3-	4-	5-	6-	7-	8-	9-	10-	11-	12-
	1 2 3 4 P A	15	3	11	1				12	3							
1 2	3 4 P A	15	3	11	0	0	1	4	11								
1 2 3	4 P A	15	3	12					15								
1 2 3 4	P A	14	3	6	2	2	1		1	3	6	4					
1 2 3 4 P	A	15	3	7	2	2	1			3	2	3	3	2	1	1	1

*Numbers show larval stages ; P=pupa ; A=Adult.
**Dissected 10 days after emergence.
***I unit : 10μ

the conditions with photoperiod of 16 hours and temperature of 27°C and thereafter transferred into the conditions with photoperiod of 10 hours and temperature of 21°C (Table 2).

When the period of exposure of mosquitoes to short photoperiod was longer, diapausing females which are indicated by small-sized first follicles were larger in number, and at the same time there was a tendency that the number of degenerate follicles became smaller.

4. *Follicular degeneration in unfed females which emerged under outdoor conditions.*

Abnormal ovarioles and normal ones were counted with unfed females having been reared

Table 3. Degeneration of first follicles in unfed females of *Culex tritaeniorhynchus* which were reared as adults from lst instar larvae and kept under outdoor conditions

Date of emergence	Date of dissection	No. dissected	No. females with degenerate follicles of following percentege of					
			0	-20	-40	-60	-80	-100
Sep. 4-5	Sep. 15	10	2	7	1			
	Oct. 31	10		1				9
	Nov. 27	7						7
	Dec. 22	5						5
Sep. 16-18	Sep. 28	10		10				
	Nov. 27	10		3	2	2	1	2
	Dec. 12	10	3	6	1			
	Feb. 2	3	1	2				
Sep. 26-28	Oct. 10	10		8	1	1		
	Nov. 27	7		4	3			
	Feb. 21-26	3		1	2			
Oct. 5-30	Nov. 27	30	1	27	2			
	Feb. 6-26	20	6	11	3			
	Mar. 1-31	18	4	14				

as adults from September to October and kept under outdoor conditions (Table 3).

Most ovarioles had degenerate follicles (Photo. C), when females having emerged in early September were reared for a month or more. On the contrary, in females having been reared as adults after middle September when the day-length becomes shorter, only a small number of ovarioles had degenerate follicles, of which morphology was as given in Photo. B, even when females were bred for about 5 months after emergence.

In this species, diapausing females with small follicles emerge after middle or late September (Kawai, 1969; Oda and Wada, 1973). From this fact and the present result, it will be said that first follicles commonly degenerate in gonoactive females which are kept for a long time without being allowed to take blood meal, but in diapausing females they degenerate only in a very low rate.

5. *Change in gonoactivity in females which emerged in early September.*

As mentioned above, gonoactive females having emerged in early September commonly form degenerate follicles. However, as it is not known whether females with many degenerate follicles can take blood meal and develop mature eggs, we examined the feeding activity, the rate of egg formation and the occurrence of follicular degeneration with females which emerged in early September. The results are presented in Table 4.

Table 4. Change in gonoactivity of *Culex tritaeniorhynchus* females which emerged in early September and were kept under outdoor conditions

Date of blood feeding	Blood feeding			Mature egg formation	
	No. exposed	No. fed	%	No. with eggs	%
Sep. 18	30	15	50.0	13	86.7
Sep. 28	30	15	50.0	13	86.7
Oct. 6	20	9	66.7	6	66.7
Nov. 11	11	0	0.0	—	—
Dec. 5	5	0	0.0	—	—
Dec. 16	5	0	0.0	—	—

Females kept without allowing to feed on a chicken until early October took blood meal actively, when given a chance, and formed mature eggs. However, females kept for a longer time became inactive in blood feeding. Five females which had survived until December were held for 10 days under conditions with 16 hour day-length and 25°C temperature, and they were permitted to feed on a chicken. Since the females did not take blood meal, they were dissected and it was found that most of the first follicles degenerated and most of the next follicles were of small size and in stage No2. These abnormal ovarioles represented the similar morphology to the abnormal ovarioles in unfed females which emerged in early September and were reared till late October (Photo. C and Table 3).

6. Change in size of first follicles in diapausing females which were kept under outdoor conditions.

We measured the size of first follicles in diapausing females which emerged in late October and were kept till various times up to March under outdoor conditions. The results are shown in Table 5.

Table 5. Developmental states of first follicles in unfed females of *Culex tritaeniorhynchus* which were reared as adults in October and kept under outdoor conditions

Date of dissecion	No. dissected	Follicular size*							Developmental stage of follicles
		3-	4-	5-	6-	7-	8-	9-	
Nov. 27	20	5	10	5					N
Feb. 19	5		1	3	1				N, Ia
Feb. 26	15		7	5	3				N, Ib
Mar. 4	15		3	3	5	3	1		N
Mar. 31	4		1	0	0	0	1	2	N, Ib

* 1 unit : 10μ

First follicles were small in size till February but they became large in March. This means that females were activated from the state of diapause. On the other hand, the rate of degenerate follicles did not change during this period of activation (Table 3). Accordingly, it seems that first follicles do not degenerate commonly in early spring under outdoor conditions.

7. Follicular degeneration in diapausing females which were kept under experimental conditions with long photoperiod and high temperature.

Even at the time of activation from the state of diapause, first follicles did not degenerate as stated in the above (see Table 3 and Table 5). However, we assumed that first follicles may degenerate in a high rate if the activated females are kept for a long time. Therefore, we performed the following experiment.

Females were reared as adults from the lst instar larvae under the conditions with short photoperiod of 10 hours and low temperature of 21°C, and held for 10 days following emergence under the same conditions. The females had small follicles and did not take blood meal, that is, mosquitoes were clearly in diapausing state. These diapausing females were transferred into the conditions with long photoperiod of 16 hours and high temperature of 25°C to activate them from diapausing state and kept for 20 days.

Ten females were examined for the numbers of abnormal ovarioles and normal ones. Only in one female, most of first follicles were found to be degenerate, and their current follicles were in stage N. In the remaining 9 females, about 10% of follicles were degenerate.

8. Follicular degeneration in unfed nulliparous females collected in the field.

With unfed nulliparous females which were collected at pigsties in middle September and kept for about 3 months under outdoor conditions, and with those which were caught in

late March and early April, numbers of abnormal ovarioles and normal ones were examined with the results shown in Table 6.

Table 6. Degeneration of first follicles in unfed nulliparous females of *Culex tritaeniorhynchus* which were collected at pigsties in middle September and thereafter were kept till December under outdoor condtions, and in those which were collected in the same pigsties in late March and early April

Date of collection	Date of dissection	No. dissected	No. females with degenerate follicles of following percentage of					
			0	-20	-40	-60	-80	-100
Middle Sep.	Middle Sep.	20	6	11	2		1	
	Middle Nov. -Early Dec.	30						30
Late Mar. -Early Apr.	Late Mar. -Early Apr.	38	15	21	2			

Abnormal ovarioles were small in number, when females were dissected immediately after collection in middle September. The morphology of the abnormal ovarioles was as given in Photo. B. However, when these females were maintained for about 3 months, most of first follicles degenerated, and abnormal ovarioles were very often represented by the morphology shown in Photo. C.

In late March and early April, 39 females were collected in the field. Among them, 38 females were nulliparous and had about 25% abnormal ovarioles. They generally showed the morphology given in Photo. B. One remaining female was parous. From these results, it may be suggested that gonoactive females which emerge in autumn will usually come to feed on animals at a comparatively early time following emergence and will not survive till next spring in an unfed state.

Morphology and rate of abnormal ovarioles were scarcely different between gonoactive females caught in early spring and diapausing females reared under outdoor conditions, as shown in Table 3 and Photo. B. These facts imply that most of overwintering females of the present species are composed of diapausing ones.

DISCUSSION

Yajima (1970) reported that ovarioles with degenerate first follicles and second follicles in stage No 2 occurred in nulliparous females of *Culex tritaeniorhynchus* which had been reared as adults under laboratory conditions, and he called this degenerate follicle "false dilatation". In the present experiment, we also could often observe abnormal ovarioles as Yajima(1970) noted, in gonoactive unfed females as well as in gravid ones. Such ovarioles as Yajima (1970) noted (Photo. B.) occurred in a low rate as early as 3 days after emergence under the condition with 16 hour photoperiod and 28°C temperature. Accordingly, it will be

estimated that some first follicles degenerate shortly after emergence and their current follicles do not develop to mature eggs, even after females take blood meal.

In addition to this type of the degenerate first follicles (as in Photo. B) as written above, such degenerate first follicles as given in Photo. C were commonly found, when gonoactive females of the present species were kept for a month or more without being allowed to feed on animals. The same phenomenon is observed also in unfed females of *Armigeres subalbatus* (Oda et al., 1976). Since unfed nulliparous females with many degenerate follicles in both mosquito species were scarcely caught in the field, it can be said that in these mosquitoes, gonoactive females feed on animals in short time after emergence in nature.

The current follicles of abnormal ovarioles in unfed females were generally 50μ or less in size in our experiments. In parous females, the current follicles of normal ovarioles are usually about 60μ or more (Kawai, 1969). Therefore, it can be said that the current follicles of parous females are larger than the current follicles of abnormal ovarioles in unfed nulliparous females.

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コガタアカイエカの未吸血未経産雌の濾胞の退化

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種々の条件下で得られたコガタアカイエカの未吸血未経産雌について濾胞の退化の状況を調査した．9月上旬頃に屋外条件下で羽化した吸血活性雌を長期間吸血させずにおくと，大部分の第1濾胞は退化するが，9月中旬頃以降の短日長下で羽化した吸血不活性雌，いわゆる休眠雌では，長期間飼育しても第1濾胞は高頻度に退化することはない．秋及び初春に畜舎で採集した未吸血未経産雌には，多数の退化濾胞を持ったものはほとんどなかった．それ故，本種の吸血活性雌の濾胞の退化は実験条件下で普通に起るが，自然界では高率に起るとは思われない．

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