ALTERATIONS IN FECUNDITY IN THE SEGMENT OF A HOUSEFLY (*MUSCA DOMESTICA* L.) POPULATION WHICH SURVIVES EXPOSURE TO DIELDRIN

by

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A THESIS

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MASTER OF SCIENCE

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INTRODUCTION

Since the appearance of resistance of house flies to chlorinated hydrocarbon insecticides, considerable work has been done on this problem. This work has been primarily directed toward determination of the degree of resistance developed, and whether or not resistance to one insecticide might be carried over to another insecticide.

Occasionally it has been observed that an increase in the population of the house flies and certain other insects may have accompanied the establishment of resistance, but no work other than that of Knutson (1953) was known to have been reported on this subject.

This investigation was concerned with the effect of a single treatment with an insecticide on the reproductive rate of the first filial generation of house flies.

Previously Knutson (1953) reported that Drosophila melanogaster Meigen, surviving dieldrin exposure, produced 5.8 per cent more adults than the control or check flies. Subsequently he suggested that this phenomena may occur in other Dipterous insects of medical importance. He also cited a comprehensive review of the literature which had dealt with this aspect. However, this has been very meager and all was done with systemic poisons such as stomach poisons and gases rather than with contact poison.

METHODS AND TECHNIQUES

The NAIDM laboratory strain of house fly was used in this investigation. This is a strain which has no history of insecticide exposure and is a standardized strain designated as the experimental subject for use in the Peet-Grady method (Soap, Blue Book, 1950, Anon). Initially, pupae were placed in small screenwire rearing cases and the adults allowed to emerge.

Within the first twelve hours after emergence the sexes were separated and kept in separate cages in order to prevent mating as shown to be a satisfactory procedure by Dunn (1923).

To facilitate handling, the adult flies, while sexing, were transferred from the rearing cages by a rapidly moving air stream to one quart cardboard cylindrical cartons provided with screened ends. These cartons were then placed in a battery jar into which a stream of carbon dioxide was passing continuously, for immobilization of the flies as described by Williams (1946). The immobilized flies were then transferred from the cardboard cartons to a Buchner funnel. A gentle flow of carbon dioxide through the funnel kept the flies immobilized during the actual separation of the sexes.

The flies were then placed in quart glass jars with a screen top and were immediately supplied, and daily thereafter, with small cups containing cotton soaked with diluted milk. A 40 per cent formalin solution was added at the rate of 1/1500 to delay souring of milk.

When the flies were three to four days old, one lot was retained without treatment as a check or control and the other lot was treated with dieldrin. This insecticide was applied to the thorax of the fly by calibrated micro-loop while immobilized with carbon dioxide at dosage of two microgm./gm. of flies, in acetone solution, which produced an average mortality of 60 per cent in the females and 87 per cent in the males. Topical application was used to assure that each fly received an identical dose of the insecticide, so that the more susceptible flies would be killed.

The treated flies were held for two days following actual topical application of the insecticide, until all then appreciable mortality had occurred. The surviving flies were separated into twenty replicates consisting of ten females and six males in each lot except in a few cases when a few flies escaped, and these lots were placed in quart glass jars with a screen top. The untreated flies were divided into lots and placed in jars in the same manner as the treated flies and served as a check or control.

The flies were kept and the progeny reared in a separate room held at a temperature of 80° F. and about 50 per cent relative humidity. Each jar was supplied daily with a fresh paper cup containing cotton soaked in milk which provided sufficient food and also served as an oviposition medium. These paper cups containing eggs were removed daily to other jars, with screen top, containing NAIDM standard larval media. A small quantity of tap water was added to the paper cups before they were put in the larval media in order to secure sufficient moisture for egg hatching and also to prevent pupation in the cotton.

The rearing jars were held for a sufficient time until all adult emergence (F_1 generation) had occurred. Thereafter the resulting adult flies were counted and the number recorded. The resulting data were a daily record of the number of offspring produced by each lot of virtually ten treated females and ten untreated (check) females, each lot being replicated twenty times.

The curve in Fig. 1 has been smoothed by application of the formula $\frac{a + 2b + c}{4}$, when "b" is the count being smoothed, and "a" and "c" represent the immediately preceding and succeeding counts, respectively.

RESULTS AND DISCUSSION

The data summarized in Table 1 and Fig. 1 show that the 187 females which survived dieldrin treatment produced a total progeny of 10,470 flies during their life time. The same number of control flies produced a total progeny of 9,141 flies; this represents a 14.5 per cent greater progeny in dieldrin-treated flies. (The number of progeny is relatively low in both groups because neither was allowed to mate until five to six days of age.) At the end of the first week the total number of progeny in the control group exceeded the total number of dieldrin-treated group by 10.9 per cent, as evidenced by the production of 5,818 flies by the control group and 5,243 flies by the treated group. The fact that the control flies produced more progeny during this period than the dieldrin-treated flies is not unexpected, since the effects of exposure to sublethal amounts of a poison might be expected to exhibit some toxic effect with accompanying reduction in normal reproduction until recovery was attained.

During the second week the trend of reproductive rate underwent a reversal. The total number of progeny of the dieldrin-treated flies and that of the control was 3,192 and 2,264 respectively, an increase in progeny of the dieldrin-treated flies over the control flies of 41.0 per cent. It was apparent that much or all of the toxic effects of the insecticide exposure had worn off by this time.

During the third week the trend of reproductive rate of the dieldrin-treated flies maintained a higher level and reached a pronounced peak, while the control flies failed to form such a peak. The actual number of progeny during this week was 1,509 and 660 flies for the dieldrin-treated flies and the control flies, respectively; this represents a 128.6 per cent increase in progeny of the dieldrin-treated flies over the control flies.

The trends during the fourth week were similar to that during the previous week, but the peak of progeny production of the dieldrin-treated flies was less pronounced. The actual number of progeny was 435 and 289 flies for the dieldrin-treated flies and the control flies, respectively, representing a 50.5 per cent increase in progeny of the dieldrin-treated flies.

In the fifth week the number of progeny of the control flies (115) actually exceeded that of dieldrin-treated flies (82); however, the total numbers were very small compared with those of previous weeks and therefore had little effect in the total progeny produced during the entire life span.

Table 1 shows that the dieldrin-treated group gave rise to a substantially greater number of progeny than the untreated flies. This difference was figured to be 14.5 per cent. Knutson (1953) also found that Drosophila melangaster Meigen surviving dieldrin exposure produced more progeny, but he found only a 5.8 per cent greater number of adults as compared to control flies.

Now under investigation as a Ph.D. thesis is the determination of whether increased longevity or life span of the dieldrin-treated flies has any influence on the greater reproductive rate; or whether the increase is merely the result of a higher reproductive capacity per fly per day. Studies also in progress include a determination of whether or not this increased reproductive rate is carried over to subsequent generations.

SUMMARY

Laboratory studies of the reproductive rate of the house fly, Musca domestica L., were conducted to determine the effect of a single treatment with dieldrin by topical application at a concentration of two microgm./per gram of flies, which produced a mortality of 60 per cent in the females and 87 per cent in the

males. Flies were separated to sexes and were treated when three to four days of age. Two days later, after substantially all mortality resulting from insecticide exposure has occurred, the surviving flies were allowed to mate. Daily records were kept of number of adult progeny. Flies which survived dieldrin treatment produced a total of 14.5 per cent more progeny than untreated (check or control) flies. During the first week the control flies actually produced more offspring than the treated flies, probably because the effects of exposure to the insecticide had not worn off. The treated flies out-produced the controls during the second, third and fourth weeks, attaining a pronounced peak of progeny production during the third week.

Table 1. Number of progeny produced by each lot of dieldrin-treated and untreated (control) house flies. Zeros indicate that parents continued to live but no progeny were produced.

Days following mating	Lot No. 1		Lot No. 2		Lot No. 3	
	Treated	Control	Treated	Control	Treated	Control
1	55	85	19	0	40	61
2	7	31	0	109	0	163
3	31	0	79	22	19	34
4	234	2	59	40	0	223
5	0	9	0	13	0	4
6	45	30	263	32	10	223
7	0	0	0	0	0	8
8	26	80	12	9	52	2
9	80	0	0	0	0	29
10	0	15	6	0	0	9
11	13	0	0	0	0	2
12	0	17	27	0	0	16
13	51	0	0	0	0	2
14	27	0	10	0	0	0
15	0	1	0	6	11	18
16	19	15	0	2	0	6
17	0	0	0	1	0	16
18	0	0	0	0	0	13
19	2	0	0	0	0	28
20	13	2	112	23	0	0
21	0	0	0	0	0	15
22	0	0	7	0	0	1
23	0	0	0	0	0	0
24	0	0	0	0	0	10
25	0	0	0	0		0
26	0	0	0	0		0
27	0	0	0	0		0
28	0	0	0	0		0
29	0	0	0	0		0
30	0	0	0	0		0
31	0	0	0	8		20
32	0	0	0	0		0
33	0		0	0		0
34	0		0	0		0
35	0					

Table 1 (cont.)

Days following mating	Lot No. 4		Lot No. 5		Lot No. 6	
	Treated	Control	Treated	Control	Treated	Control
1	23	7	8	52	1	1
2	24	40	27	3	11	30
3	118	304	25	22	23	63
4	0	53	90	200	150	263
5	0	10	91	4	0	3
6	247	217	0	79	122	745
7	0	15	247	17	0	93
8	55	290	85	0	107	15
9	15	2	0	44	0	126
10	0	12	217	0	114	45
11	157	64	0	16	0	0
12	25	0	75	0	2	0
13	0	0	0	0	19	0
14	104	16	39	6	11	2
15	34	12	0	0	0	9
16	46	0	0	0	0	0
17	19	2	10	0	24	3
18	90	0	47	12	4	5
19	8	0	7	20	13	3
20	25	10	7	0	0	0
21	0	0	7	0	0	7
22	34	0	28	20	0	2
23	0	0	0	0	0	0
24	0	0	0	0	0	0
25	59	0	0	4	0	0
26	0	0	0	0	0	0
27	0	0	0	0	0	0
28	0	0	0	0	0	0
29	0		12	21	0	0
30	0		0	0	0	0
31	0		0	0	0	0
32	0		0	0	0	
33	0		0		0	
34	0		0			
35			0			
36			0			

Table 1 (cont.)

Days following mating	Lot No. 7		Lot No. 8		Lot No. 9	
	Treated	Control	Treated	Control	Treated	Control
1	0	42	0	0	0	14
2	220	67	28	31	0	7
3	13	6	8	3	43	0
4	0	36	0	0	0	0
5	78	97	100	163	93	0
6	0	5	38	25	0	0
7	7	16	45	21	7	0
8	45	3	38	238	0	0
9	153	9	35	6	65	0
10	0	0	41	0	3	0
11	76	0	0	6	0	0
12	1	0	14	0	0	
13	9	0	27	0	0	
14	2	7	34	0	1	
15	0	0	0	19	0	
16	5	0	0	30	29	
17	0	1	1	0	0	
18	0	0	7	0	1	
19	2	0	24	0	5	
20	5	0	0	0	81	
21	0	0	0	34	0	
22	0	2	0	0	0	
23	0	0	0	3	23	
24	0	0	0	0	0	
25	0	0	0	0	0	
26	0	0	0	0	0	
27	0	0	63	0	56	
28	0	0	0	0	0	
29	0	0	0	0	0	
30	0	0	0	0	72	
31	0	0	0		0	
32	0	0	0		0	
33	0		0		0	
34			0		0	

Table 1 (cont.)

Days following mating	Lot No. 10		Lot No. 11		Lot No. 12	
	Treated	Control	Treated	Control	Treated	Control
1	0	17	0	0	0	24
2	0	114	0	126	8	31
3	151	56	0	7	58	13
4	0	0	13	0	0	36
5	21	77	0	5	124	27
6	5	40	29	29	0	0
7	176	0	0	12	0	2
8	27	12	0	0	94	59
9	41	15	0	0	0	7
10	57	10	0	5	17	0
11	18	0	0	0	0	3
12	0	22	0	0	8	0
13	76	0	0	2	93	0
14	0	0	14	0	11	0
15	24	4	0	0	0	0
16	22	0	0	0	9	0
17	44	0	0	0	0	0
18	14	20	0	0	25	0
19	101	0	0	0	26	0
20	7	1	0	0	0	4
21	14	0	0	0	0	0
22	0	1	0		10	0
23	0	3	0		0	0
24	0	0	0		0	0
25	0	0	0		0	0
26	0	17	0		0	0
27	35	0	0		0	32
28	0	0	0		0	0
29	0	0	0			0
30	0	0	0			0
31	0	0				0
32	0	0				0
33						0

Table 1 (cont.)

Days following mating	Lot No. 13		Lot No. 14		Lot No. 15	
	Treated	Control	Treated	Control	Treated	Control
1	0	0	7	11	0	0
2	0	25	3	77	7	31
3	22	13	3	0	0	24
4	5	35	0	14	80	2
5	0	6	220	16	0	58
6	0	53	0	40	24	24
7	130	0	64	34	90	0
8	0	25	2	33	30	69
9	33	2	51	19	0	16
10	13	0	0	0	0	0
11	0	0	1	23	0	51
12	0	0	0	0	0	0
13	4	0	45	26	0	4
14	8	0	0	0	0	0
15	0	0	0	0	20	22
16	0	0	0	2	0	0
17	24	0	10	42	0	0
18	15	5	0	22	0	0
19	0	0	0	1	0	0
20	0	0	0	5	38	0
21	0	14	0	21	0	0
22	24	0	0	0	0	0
23	0	0	2	0	0	0
24	0	0	0	0	15	0
25	0	0	0	0	0	0
26	0	0	0	0	0	2
27	0	0	0	0	0	0
28	0	0	0	0	0	0
29	0	0	0	0	0	0
30	0	0	0	0	0	
31	0	0	0	0		
32	0	0	0	0		
33	0					

Table 1 (cont.)

Days following mating	Lot No. 16		Lot No. 17		Lot No. 18	
	Treated	Control	Treated	Control	Treated	Control
1	3	30	0	3	0	30
2	0	0	23	51	48	0
3	24	63	94	13	0	15
4	29	0	18	31	0	27
5	101	37	107	173	0	18
6	0	0	47	0	0	31
7	21	47	72	0	73	62
8	8	130	121	269	0	30
9	0	38	88	1	0	12
10	0	3	0	0	40	7
11	0	29	33	0	0	22
12	0	7	19	9	1	0
13	0	0	0	0	0	0
14	0	7	0	0	0	32
15	0	29	11	0	29	0
16	0	0	0	0	0	0
17	11	10	11	17	0	6
18	0	0	50	0	54	4
19	0	28	0	21	0	43
20	0	23	0	0	0	0
21	0	8	0	0	2	17
22	0	0	0	12	0	3
23	0	11	0	0	7	0
24	0	0	0	26	0	0
25	0	8	0	0	0	8
26	0	0	0	0	0	0
27	0	0	0	0	5	0
28	0	11	0	11	0	6
29	0	0	0	0	0	0
30	0	0	0	0	0	0
31	0	0		46	0	0
32	0	0		0		0
33		0		0		0

Table 1 (concl.)

Days following mating	Lot No. 19		Lot No. 20	
	Treated	Control	Treated	Control
1	0	2	0	47
2	48	45	33	8
3	0	12	140	14
4	45	39	26	37
5	36	10	116	3
6	60	4	0	0
7	0	12	31	2
8	0	0	11	92
9	221	0	0	0
10	11	12	14	0
11	22	0	25	0
12	22	0	13	2
13	0	0	21	0
14	6	6	12	0
15	16	0	0	0
16	53	0	13	0
17	0	0	0	7
18	95	0	15	0
19	0	0	27	0
20	20		12	0
21	2		0	0
22	0		0	3
23	17		0	0
24	0		0	39
25	5		0	0
26	50		0	0
27	0		0	0
28	0			20
29	0			0
30	0			0
31	0			0
32	0			0
33	0			0

Table 2. Relative number of progeny produced by 187 dieldrin-treated and 187 untreated (control) house flies.

Number of days following mating	Total number of offspring			
	Control		Treated	
	Daily	Weekly	Daily	Weekly
1	426		161	
2	1089		485	
3	560		856	
4	977		746	
5	660		1037	
6	1763		845	
7	343	- 5818	1063	- 5243
8	1109		687	
9	554		728	
10	204		613	
11	222		337	
12	77		230	
13	24		294	
14	64	- 2264	303	- 3192
15	107		172	
16	44		182	
17	128		173	
18	81		417	
19	151		325	
20	68		197	
21	32	- 660	43	- 1509
22	75		98	
23	14		25	
24	42		39	
25	20		59	
26	58		5	
27	32		204	
28	28	- 239	5	- 435
29	21		12	
30	20		72	- 82
31	74	- 115	0	
32	0			
33				
34				
35				
36				
37				
Totals	9141		10470	

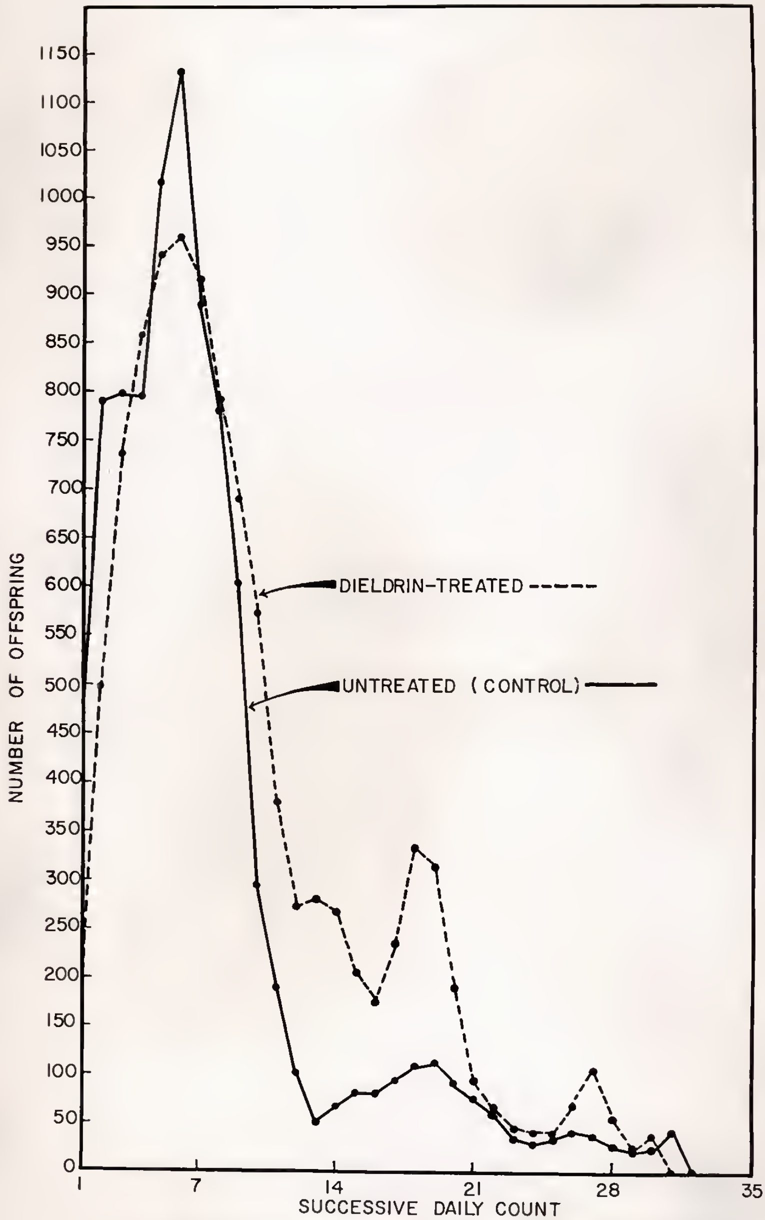

Figure 1. Offspring produced by 187 Musca domestica exposed to dieldrin and 187 controls, on successive days throughout productive life.

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The writer wishes to express his gratitude and appreciation to Doctor Herbert Knutson, Head of the Department of Entomology, for suggesting this problem and for the supervision of this work and for his unfailing help and encouragement. Appreciation is also expressed to all the staff of the department for aid and assistance. The help of Mr. M. Abdulla is also sincerely appreciated.

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Numerous studies have been conducted during recent years on resistance of house flies to chlorinated hydrocarbon insecticides. However, this work has been primarily directed toward determination of the degree of resistance developed, and whether or not resistance acquired to one insecticide might be carried over to another insecticide. Occasionally it has been observed that an increase in the population of ~~the~~ house flies and certain other insects may have accompanied the establishment of resistance. However, no work other than that of Knutson, working on Drosophila, is known to have been reported, in which he found that Drosophila which survived dieldrin exposure produced 5.3 per cent more offspring than the controls.

This investigation was concerned with the effect of a single treatment with an insecticide on the reproductive rate of the first filial generation of house flies.

The NAIDM laboratory strain of house fly with no history of insecticide exposure was used. Initially, pupae were placed in small screenwire rearing cages and the adults allowed to emerge.

Within the first twelve hours after emergence the sexes were separated and kept in separate cages in order to prevent mating. To facilitate handling, the adult flies, while sexing, were transferred from the rearing cages by a rapidly moving air stream to one quart cardboard cylindrical cartons provided with screened ends. These cartons were then placed in a battery jar into which a stream of carbon dioxide was passing continuously, for immobilization of the flies, after which they were transferred from the cardboard cartons to a Buchner funnel. A gentle flow of carbon dioxide through the funnel kept the flies immobilized during the actual separation of the sexes.

The flies were then placed in quart glass jars with a screen top and were immediately supplied, and daily thereafter, with small cups containing cotton soaked with diluted milk.

When the flies were three to four days old, one lot was retained without treatment as a check or control and the other lot was treated with dieldrin. This insecticide was applied to the thorax of the fly by calibrated micro-loop while immobilized with carbon dioxide at dosage of 2 microgm./gm. of flies, in acetone solution, which produced an average mortality of 60 per cent in the females and 87 per cent in the males. Topical application was used to assure that each fly received an identical dose of the insecticide, so that the more susceptible flies would be killed.

The treated flies were held for two days following actual topical application of the insecticide, until all then appreciable mortality had occurred. The surviving flies were separated into twenty replicates consisting of ten females and six males in each lot and these lots were placed in quart glass jars with a screen top. The untreated flies were divided into lots and placed in jars in the same manner as the treated flies and served as a check or control.

Rearings were made at 80° F. and about 50 per cent relative humidity. Each jar was supplied daily with a fresh paper cup containing cotton soaked in milk which provided sufficient food and also served as an oviposition medium. These paper cups containing eggs were removed daily to other jars, with screen top, containing NAIDM standard larval media. Tap water added to the paper cups insured sufficient moisture for hatching and also prevented pupation in the cotton. Following pupation and emergence the resulting F_1 adult flies were counted, resulting in a daily record of the number of offspring produced by each lot of ten treated females and ten untreated (check) females, each lot being replicated twenty times.

The 187 females which survived dieldrin treatment produced a total progeny of 10,470 flies during their life time, while the same number of control flies produced a total progeny of 9,141 flies; this represents a 14.5 per cent greater progeny in dieldrin-treated flies. (The number of progeny is relatively low in both groups because neither was allowed to mate until five to six days of age.)

At the end of the first week the total number of progeny in the control group was 5,818 as compared to 5,243 in the treated group, an increase of 10.9 per cent over the treated group, which is not surprising, since the effects of exposure to sublethal amounts of a poison might be expected to exhibit some toxic effect with accompanying reduction in normal reproduction until recovery was attained.

During the second week the total number of progeny of the dieldrin-treated flies and that of the control was 3,192 and

2,264 respectively, an increase in progeny of the dieldrin-treated flies over the control flies of 41.0 per cent. It is apparent that much or all of the toxic effects of the insecticides had worn off during the first week.

During the third week the trend of a higher reproductive rate in the dieldrin-treated flies continued, and a pronounced peak was reached, which did not occur in the control flies. Total progeny were 1,509 and 660 flies for the dieldrin-treated flies and the control flies, respectively, a 128.6 per cent increase in progeny of the dieldrin-treated flies.

Trends during the fourth week were similar to that during the previous week, but the peak of progeny production in the dieldrin-treated flies was less pronounced. Progeny totaled 435 and 289 flies for the dieldrin-treated flies and the control flies, respectively, representing a 50.5 per cent increase in progeny of the dieldrin-treated flies.

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