[1943]

(from Institute of the German Brain Research Association, Neustadt/Schwarzwald) On the Production and Meaning of Metamorphosis-Promoting Hormones During the Larval Development of *Drosophila*

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With 55 illustrations and 9 tables

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A. Introduction

So far we know from individual representatives of different groups of insects that as a consequence of elimination of the corpus allatum in early larva stages, by extirpation, decapitation or ligature, metamorphosis will occur comes prematurely. WIGGLESWORTH (1934, 1936 and 1940) highlighted this finding with the hemipteran *Rhodnius*, PFLUGFELDER (1937) with the Orthopteran *Dixippus*, BOUNHIOL (1937, 1938) and FUKUDA (1940) with Lepidoptera, in particular with *Bombyx*. In some groups of insects at least under the experimental conditions mentioned above metamorphosis-promoting hormones are thus formed already in early larval stages.

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In the opinion WIGGLESWORTH (1940) the latter is now also the case in the normal development of the heterometabolous land bug *Rhodnius*. The same metamorphosis hormone promotes growth in each larval instar and, thereby, the larval molts as well, and later causes the imaginal (adult) molt. The larval character of the early larval stages would be stipulated on the other hand solely through the additional effect of the corpus allatum-hormone.

Recently also FUKUDA (1940) also came to a similar conveyance from the results ligation experiments on the caterpillar of the holometabolous silk spinners (*Bombyx mori*). For the larval molt of *Bombyx* likewise the prothoracic glands (after the author assigned to its hormones the formation of the pupal molt) are necessary beside the active substances of the corpora allata.

Thus, after both authors used representatives of two different groups of insects to examine the hormones of the imaginal molt and/or pupal molt it is that these hormones are of substantial importance for the normal larval development.

In the following, studies with *Drosophila hydei* are presented, which aim to gradually clarify the relevant conditions for *Drosophila*. There are four questions on which a position is taken.

1. Can ring glands of the first and second larva instar form those hormones which promote the pupation and imaginal differentiation in *Drosophila*?

2. Is - in the case of a positive answer of the question 1 - the functional behavior of the ring glands during the larval stadia steady, or primarily periodically changing and then particularly adapted to the larval development?

3. Can the developmental process which normally take place within the larval stage be promoted likewise by implanted ring glands?

4. To the affirmation of the question 3, is the larval developmental process driven by the same ring gland cells as with the puparium formation that uses metamorphosis processes?

The conclusions resulting themselves from the answering of these questions will be elaborated in the Discussion.

B. Materials and Methods

The investigations were accomplished with the same wild type *Drosophila hydei* (Italy), as those that were raised by me to make previous findings (1942b). All studies were made at a rearing temperature of 25° C. The application of string ligations and implantation technology are already described elsewhere (1942b).

The larval age was counted again from the time of eclosion from the egg, whereby the larvae ecdysing during one period of two hours were used as being of the same age. Apart from the larval age also always still considered was the larval instar, for example if the 2 Day 21 hr. old larvae of the second larva instar were to be used, then it was carefully examined whether these larvae also still appeared to show no molt. For this the larvae themselves were examined on white underground in a water drop for first signs coloration of the new mouth hook. On the other hand if the larvae should be in the molt stage, then larvae were selected which had developed, alongside the old mouthhooks, the new mouthhooks and hind spiracles that were already colored. The first molt took place for instance at the age of 1 day 15 hours, the second at the age of 2 days 22 hours; this value can be regarded only as an approximate value, since on different days of study the molting time could vary by some hours. Furthermore, in molting itself the male larvae are regularly more early than the female larvae from the same breeding cups.

The small size of the ring glands of younger developmental stages, as well as the implantion of pieces of ring gland, required the use extremely thinly polished little knives, which was accomplished by loops of common needles. Because of the long duration of the preparation only a small series of tests could be accomplished on the same day, on the average 4-8 cases. The series shown in the tables always consist therefore of the sum of many small series of tests. Through this random errors by remaining unknown external factors to be to a large extent controlled for. Furthermore there was always at least with two of such series of tests on the same day, which should be compared with one another. Thus, e.g., I implanted ring glands of donor larvae, which were at the end of the second stadium, exclusively on days on which I transplanted also ring glands of the early second stage at the same time.

The length- and width diameter of implanted eye antennal imaginal discs and testicles were measured under Ringer solution with an ocular micrometer. A micrometer unit corresponded to 17 microns.

For the histological investigation the organs were fixed in Sanfelice and the 4 micron thick sections with HEIDENHAIS Eisenhaematoxylin and Saeurefuchsin as the counterstain color.

I thank Mrs. RUTH HASSLER in the best way for the preparation of the histological preparations and Fraulein LUTSE ROSSI for its assistence with the transplantions.

C. Experimental Part

1. Promotion of metamorphosis processes by ring glands of the first and early second larval stage

As well known, HADORN (1937) first showed that the ring gland of *Drosophila* delivers the hormone for the formation the pupal case or puparium. Investigations of NYST $(1941)^1$ and my own (1942b) continued to show then that the ring gland also drives operative pupating processes internally within its self in first day of the pupae.

¹) In the case of my minor publication of the year 1942(b), the findings of NYST were unfortunately not yet well-known, so that they did not experience my coming acknowledgement in my remarks at that time.

I found the same for the "imaginal differentiation" following that stage. With all these studies only ring glands of the third, i.e. last, larva instar were transplanted.

It begs question as to how far ring glands of the first and second larval instar can promote the same processes.

a. Pupating Processes

Regarding the pupation processes the summary in the table for the tests of series C and D give an answer to Question 1 (pg. 402). In the Series C tests ring glands of the early second larval stadium, and in series D of the first larva instar, were implanted into isolated abdomens of 4 Day 21 hr. old larvae. Since such abdomens cannot on their own form a puparium, (see control series the Table 1), any occurrence of pupating features must be attributed to the delivery by metamorphosis hormones by the implanted ring glands.

As Table 1 shows now, first, in Series C after three days 85% of the host abdomens had formed a puparium, and in series D 80%. At the same time all 127 control abdomens, which had not received ring gland implants, had remained larval.

In the ring gland series, parallel with the external pupation features also the internal organs (intestine, fat body, gonad) of the host abdomens as well as implanted eye antenna disks showed transformations characteristic of at least the first pupal day. Since the changes in the implanted eye antennal imaginal discs resembeled those completely detailed by me previously, I refrain here from their description.

The young ring glands therefore can promote all typical pupation processes. Because these processes already appeared in one day in 16 or 13 cases (series C or D) and within this short time any change in the implanted ring glands' own development is unlikely, series C and D might be enough to prove that already ring glands of the first and second larva instar can produce pupation hormone.

It is still added that as a control, on same days were implanted the above-throatganglia of the early second larval stadium, which did not release discernable pupation characteristics (Series E); it is not thus possible at this larval stage for the *Drosophila* "brain" to also be a place of formation of pupation hormones (see in comparison WIGGLESWORTH, 1940).

b. Imaginal differentiation processes

It has already been previously described it detail by me elsewhere (1942b) that *Drosophila* ring glands implanted in ligated abdomens not only promote pupation process, but also imaginal differentiation processes. This result lets us assume at least an identity the active substances that release these processes (see moreover also page 444). It caused now the examination of whether ring glands of the first two larva stages can also in a similar way release imaginale differentiation processes, beyond pupating processes, in ligated abdomens

From BODENSTEIN (1938) we know in the higher development stages of the eye imaginal disks (stage 4 - 7 BODENSTEIN), the "imaginal differentations" are accompanyed by pigment formation, and among other things the relatively late appearing coloration of the abdominal hair.

It is first emphasized that the higher development stages of the eye imaginal discs do not necessarily do not commence after the release of the first pupating changes. Thus my own implantations (1942b) of 1-2 hours old pupal eye antennal imaginal discs showed that in ligated (thus ring-glandless) larval abdomens that the implants reached the cap stage¹, thus, the pupating stimulus had been already received prior to implantation. However the formation of the pigment stage that follows in the normal development did not occur. For a long time the combined ligation studies and transplantation studies of BODENSTEIN (1938) led to similar results concerning the ocular development and concerning the coloration into the abdominal hair in young pupae.

The few abdomens of the series C and D which survived the second day after the occurance of the first pupating features without exception now showed however these imaginal differentiations. Since such imaginal differentiations do not take place in ring-glandless isolated larva or young pupae abdomens in usual atmosphere (see moreover BODEINSTEIN, 1939b), its occurrence must be understood as consequence of the ring glands implantation.

Fig. 1 and 2 shows two abdomenal hindpieces, for which 7 days after the operation (5 days after formation of the puparia) the individual abdominal hair itself begins to fill out with color. The eye antenna implants of the same cases showed red eye pigment over the whole eye part and colored hair in the head regions (Fig. 3a, H). The ommatida were somewhat indistinct because partial rot in their structure had already occurred. For instance, they corresponded to the stage 7 of BODENSTEIN (Fig. 3b and 4) in their degree of development.

^{&#}x27;) I designated the cap stage as the stage of the eye antennal imaginal discs in which these discs, after implantation into a equivalent unligated host larva, reach at the second half of the first day of the quiescent stage of the host pupa.





- Fig. 1. Beginning of coloration of the hair in ligated abdomens of the Series C, 7 days after implantation of six 1 Day21 hr old ring glands. 30/1
- Fig. 2. Ligatured abdomen of the Series D, 9 days after implantation of eight 21 hrs of old ring glands. 30/1



Fig. 3b

Fig. 3. Ocular implant of the same case like in Fig. 1. in a: 125/1. in b: 500/1. *A* antenna, H = already colored head hair. Ocular part between arrows, at stage 7 ---.

Fig. 3a

Furthermore in both case the vasa efferentia and the sperm pump could both already be recognized and the sperm pump in both cases already clearly; the testicles not strongly connected with the execution gears had grown and in the case of Fig. 1 contained ample sperm. Furthermore in the abdomens of Fig. 2 had developed the imaginal kropf (= crop?) (Fig. 5).

The third case with ' imaginalen differentiations ' still showed no coloration of the abdominal hair; the ocular implant was at stage 5 and owned brown ocular pigment. Also here the testicles had strongly grown and contained a small quantity of seminal threads.



Fig. 4

Fig. 5

Fig. 4. Ocular implant of the case of Fig. 2. Stage 7. 500/1. Fig. 5. Formation of the imaginal crop (Kr) in the abdomen of Fig. 2. Oe = esophagus. Pr = Proventriculus. 80/1.

We see in this way that ring glands of the first and early second larva instar can, apart from the first pupating transformations, also release imaginal differentiation processes in ligated abdomens in the same way as "pupate-ripe" ring glands.

II. Different behavior of different aged ring glands

I then went to the question of different functional behavior of different aged ring glands, first by implantation of the latter in isolated abdomens of the third larval instar, and secondly by such into unligated larvae of different development stages.

a. Findings with implantation into isolated abdomens of the third larval instar

As to the comparative effects of different aged ring glands of the last larva instar, I showed in an earlier work (1942b) that with increasing age of the donor larvae there was increasing induced metamorphosis processes in the ligated abdomens of Day 5 15 hr. old host larvae (1942b, page 154, Table 4).

Thus, e.g., six ring glands in the ligated abdomens from 3 Day 15 hr. old larvae caused metamorphosis processes on the average after five days, but after an average of three days with four ring glands from 4 Day 15 hr. old larvae, and already after one day with a ring gland of a six old "pupation-ripe" larva. Thus the implantation studies with ring glands of the third larval instar entailed the faster release of metamorphosis as as the donor larvae were more close to their own pupation.

With my studies with ring glands of the first and second larva instar the implantation took place in 4 Day 21 hr old host larvae. In order to be able to better compare now the above results with ring glands of the third larva instar, with those of the first and second instar, ring glands of only 4 Day 21 hr last larva stage became transplants in a new series of tests in isolated larval abdomens.

Table 1. Release of the puparium formation into isolated 4 Day 21 hr old larval abdomens by implantation of different aged ring glands. In all series of tests, including control series, the implants were always 4 Day 21 hr old eye antenna disk implants.

3 Days 21 hr. = first half of third larval stadium.

2 Days 21 hr. = end of second larval stadium.

1 Day 21 hr. = first half of second larval stadium.

0 Days 21 hr. = second half of first larval stadium.

			Status	of the abdon	nens ope	rated on		
			1	2	3	4		% of the
Designation	Additional	Time after		hind 1-2		dead w/out	n	total
of test	implanted	operation	total	segments		apparently	columns	abdomens
series	organ	in days	pupation	pupated	larval	pupating	1-3	pupating
A	six 3 Day 21 hr	3	-	-	22	20	22	0
	old ring glands	5	3	1	3			
В	six 2 Day21 hr	3	1	_	48	34	49	2
	old ring glands							
С	six 1 Dav21 hr	1	12	4				
Ū	old ring glands	2	35	3				
		3	39	3	4	24	46	84.8
D ¹	six Dav 0 21 hr	1	5	8				
	old ring glands	2	19	4				
		3	20	4	1	4	25	80
E	six 1 Dav21 hr	3	_	-	41	52	41	0
	old brain	-				_		-
Control	-	3	-	-	127	125	127	0
		5			55			

¹) In the D series, out of especial concern to avoid injury to the very small ring glands, often the adjoining eye antennal imaginal discs were not removed.

This happened partly in studies on the same days in which also implanted were recent stage ring glands.

The results are summarized in the Tables 1—4. Here are the series of tests in which those sets in the same table were performed mostly on the same days.

Table 2. Time of the puparium formation with implantation of a 3 Day 21 hr. and/or 1 Day21 hr. old ring gland. Furthermore, 4 Day 21 hr old eye antennal disc implants were always implanted into the 4 Day 21 hr. old isolated abdomens.

3 Days 21 hr. = first half of third stadum.

1 Day 21 hr. = first half of second stadium.

			Status 1	of the abdo 2	mens ope 3	rated on 4		% of the
Designation of test series	Number and age of ring gland implants	Time after operation in days	total pupation	hind 1-2 segments pupated	larval	dead w/out apparently pupating	n columns 1-3	total abdomens pupating
F	one Day 3 21 hr old ring glands	3 4 6 8	- 2 14 18	- 1 2	53 8	25	53	0
Ĝ	one Day 1 21 hr old ring glands	1 2 3	9 14	1 3 3	1	6	18	77.8
Control	-	3 8	-	-	77 30	67	77	0

Table 3. Puparium formation after implantation only of one ring gland. Furthermore, 4 Day 21 hrs. old eyes-antennal discs and 1 Day13 hr. old brain-ocular complex were implanted into 4 Day 21 hrs of old isolated larvel abdomens.

4 Days 21 hr. = the second half of the third larva stadium.

2 Days 21 hr. = end of the second larva stadium.

1 Day 21 hr. = the first half of the second larva stadium.

1 Day 13 hr. = time of the first molt.

			Sta	atus of the a	bdomens o	operated on		% of the
Designation of test series	Additional im planted organ	Time after operation in days	total pupation	hind 1-2 segments pupated	larval	dead w/out apparently pupating	n columns 1-3	total abdomens pupating
н	one Day 4 21 hr old ring glands	1 2 3	- 7 26		49	38	75	34.7
	one Day 2 21 hr old ring glands	3	-	-	15	15	15	0
J	one Day 1 21 hr old ring glands	1 2 3	2 8 9	2 3 3	4	8	16	56.3
Control	_	3	-	_	90	101	90	0

Furthermore, still to be discussed in part III of this work are the series of tests of the Tables 3 and 4 for the brain eye antenna complexes and/or young testicles implants. Since this happened in relation to test series posed in the same way, the presence of these implants can remain unregarded in the present connection.

Table 4. Comparison of the puparium formation time with implantation of one 4 Day 21 hr or one Day 5 21 hr. old ring gland. Furthermore, 4 Day 21 hrs. old eye antennal discs were implanted into the 4 Day 21 hr. old isolated abdomens.

Designation of test series	Number and age of ring gland implants	Time after operation in days	Status 1 total pupation	of the abdo 2 hind 1-2 segments pupated	omens oper 3 larval	rated on 4 dead w/out apparently pupating	n columns 1-3	% of the total abdomens pupating
К	one 5 Day 21 hr old ring gland	1 2 3	11 29 32	- - -	1	6	33	97
L	one 4 Day 21 hr old ring gland	1 2 3	- - 14	- 2 3	14	11	31	45.2
Control	-	3	-	-	83	81	83	0

If one hand we compare in Table 1 the effect of the ring glands of the early third larva stage (Series A) to that early second (Series C) and the first larva stage (series D), it is striking that the latter ring glands in spite of their lower size show a clear metamorphosis effect already 1-2 days after the implantation, while such is still absent with ring glands of the first half of the third larva instar at the same time.

The corresponding also applies to Series F and G of Table 2, in which only single ring glands of the early third and early second larvel instar were implanted.

The delivery of metamorphosis hormones is clearly bigger therefore by ring glands of the first and early second larva instar soon after the implantation than that of the older ring glands of the early third instar.

Still made here is an interesting consideration of associated findings. From Table 2 it is evident in Series F that implantation of single 3 Day 21 hr. old ring glands caused metamorphosis changes in the host abdomen 6-8 days after the operation. It must be that an amount of metamorphosis hormone very close to that of the "pupation mature" ring gland is delivered by the implanted single ring gland after a longer stay in ligated abdomen.

It seems functional to a large extent to develop thus also a ring gland from early in the third larval stadium in the isolated abdomen, detached by their environment and their nervous

connections, that will become "pupating mature." The histological substrate of this advancement: A large increase of the nucleus and cytoplasm, as well as the appearance of vacuoles in the latter were already described and illustrated by me (1942b, Fig. 57). Whether this development of the ring gland is to be understood here as a completely autonomous procedure, however, can not be decided, because also the lymph of an isolated abdomens cannot be understood as an indifferent environment.

It can be firmly proven in addition that there are already substantial differences in the effect strength of differently aged ring glands within the second larva instar. Thus ring glands which were at the end of the second larval stadium still showed no metamorphosis effect of donor larvae on the third day after the implantation, with an exception of a single experimental larva.¹ Compare Series B of the Table 1 and Series I of Table 3 to the clear metamorphosis effect during the same days of implantation of early second larval instar ring glands (series C or J)! Besides, almost without exception the isolated abdomens of larvae of the same stage ecdysed spontaneously. A donor larva finding itself at the end of the second larval stadium thus already had the critical period for the second larval molt behind itself.

Furthermore, Table 3 informs us that even a ring gland of the middle third of the last larva stadium (Series H) does not soon after implantation yet reach the strength of effect of a significantly smaller ring gland of the early second larva stadium. The older ring glands caused metamorphosis processes 2 days after the implantation only in 9 % of the cases, while metamorphosis processes were released by the younger ring glands (J) at the same time in 11 by 16 cases (= 69%). Only a ring gland of the late third larval stadium (Series K of Table 4) reaches and/or exceeds the effect strength of the young ring glands.

We can summarize the results thus as: With implantation into isolated larval abdomens of the third instar, ring glands of the first larval instar, early second larval instar, and the late third larva instar soon after the implantation show a clear delivery of metamorphosis hormones, while at the same time such is not yet provable with ring glands of the late second larval instar and the first half of the third larva instar.

It is still noticed supplementally that also the ring glands taken at the moment of the first larval molt, 1 Day13 hrs. old larvae showed just as strong an effect as I described above for ring glands of the center of the first larva instar and/or the early second larva instar. Therefore I refrained from making a special tabular rendition of that result.

¹) supposedly because of the physiologically recent stage of the donor larva.

			c			27		0	2	u u	2	
			12		7C	1000	(100)			99	(100)	
		ay 7	œ	ours	ų	9 8				65	(98)	
Irvae		õ	4	Ť	5	3 8				64	(67)	
star La			0		Ó	<u>o 6</u>	5			22	(98)	
nd Ins	d Tim		20		ć	2 6	())			49	(74)	
Seco	dicate		15		-	+ £				34	(51)	
andsof	the Inc	ay 6	12	ņ		23				<u>0</u>	(22)	
ling Gl	J Up to	ŏ	œ	Hour						4	0	
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se of Pu	Nur	Day 5	œ	Hours				18	(1 00)			
Relea			4					14	(78)			
mature			0					6	(20)			55 rva 1
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Table		mulantad areas	iiibiaiiteu uigair		Four	Day 2 21 hour	ring glands	Four Dev 1-21 hour	ring glands			uc
		Series	number			Σ		Z	z		Control	

The supposed meaning of the differences described here in the effective strength of different age ring glands are more closely analyzed only in the Discussion.

b. Findings with implantation into unligated larvae of the second and third instar

Since the described studies uncovered a different effect of different age ring glands, it appeared of interest how far such could be proven also with implantation into unligated larvae. This study arrangement offered further for purely technical reasons still the possibility to expand the studies to larvae of the second stadia to be the host larvae.

According to the just described difference in the delivered metamorphosis hormone amounts between ring glands of the first larval instar and early second larval stadia on the one hand, and of the late second larval stadium, on the other hand, most experiments with ring glands of these age steps are included.

s of tests are summarized, in which were implanted, in val stadium, the ring glands of the late second larval y second larval stadium (Series N). Both series of tests me of the puparium formation. The puparium ced, with one exception, at approximately the normal

of puparium formation (supposed due to a younger donor larva). In comparison with the control larvae into which only Ringer solution was only injected, all 18 larvae in the series N were at least around 24 hours premature in puparium formation (compared with the earliest pupating time of control larvae). Without exception, going in parallel with this was a premature release of pupation features by in the internal organs.

In order to determine this, the prematurely pupated larvae were dissected about 14-20 hours after the first occurance of external pupation characters, thus always at a time when all control larvae were still in a larval condition. This time of 14-20 hours was selected since in the normal development the eversion of the imaginal discs and thus the formation of the actual pupal body are terminated about 14 hours after the occurance of of the puparia.

Thus 7 cases showed at this time of dissection a finished pupal body; with the remaining 11 cases leg and wing discs were out-inverted with a formed pupae abdomen. On the other hand, missing was the eversion of the eye antenna imaginal disks. Due to the similarity with the "cryptocephalen pupae" stage of normal development (see ROBERTSON, 1936) I label these pupae from now as "cryptocephal." However, in contrast to normal development, the eye antennal discs in the cryptocephalic condition substantially continue to develop. Apart from a strong growth in size the cryptocephal hosts had usually formed "pupal cuticle pigment" (see VOGT, 1942b, page 136) in the scalp district, which arises in the normal development only after eversion of the imaginal disc.

It is first most obvious to attribute this missing eversion of the eye antennal discs on a the latter not having yet a competence to react. Speaking against such an explanation



Fig. 7

Fig. 6. Side view of a normal puparia. With arrow: Basis of the front spiracles. 10/1. Fig. 7. Curved puparia of two pupae from Series N. missing the eversion of the front spiracles. With arrow: posterior end of the inside-formed pupal abdomen. 10/1

however is the fact that in my experience the process of eversion "pupate-matures itself" also in spontaneously pupating hosts. Thus, the ligated front pieces can be omitted and still the scalp pigment forms and leg and wing discs become everted. Therefore, it seems to me nearer to the situation to come back the non-appearance of the eversion in the prematurely pupating cases of the series N, as well as in the ligated front pieces, as being due to a disturbance of the muscle activity. Earlier authors (Beckner, in 1941, NYST, in 1941, among others) have already accepted such for ligated larval halves. The pupariumin sclerotization occurs, nevertheless, in the ligated larval halves without the contraction of the larval body preceding the normal development! The latter applies now strongly likewise to those prematurely induced unligated pupae ("pseudopupae" HADORN). Also it kept (when at the same time strong curvature, compare Fig. 7 with Fig. 6) the oblong form of the larva. The large distance that exists between the hind ends of the pupal abdomen forming inside the pupal case and that of the pupal case shows that with them now is really an unsatisfactory contraction of the larval body (Fig. 7, upper). Furthermore also still the imperfect eversion of the front spiracles which can be observed with the pseudopupae speaks for a disturbance of the muscle activity (see a comparison against it in Fig. 6, lower).

In this connection still one more deviation which can be briefly observed is mentioned here about pseudopupae. During the normal pupating the entire pupal puparium color looks very even, apart from the somewhat hurrying ahead area of the spiracles. With the pseudopupae and in the same way with prematurely induced pupation of ligatured abdomens, the area of the anal plate hurries ahead by the profile of its lattice structure (Fig. 8 and 10, P),





making the discoloration of the remaining parts of the pupal case become apparent prematurely. Since the remaining parts of the pupal case often do not achieve the dark red color of normally colored pupal puparia, the plate usually also still stands out by its darker color against its environment even after terminated colorization. This atypical coloring process is based probably to a large extent on the fact that a still young larval cuticle with a strongly prematurely induced pupal molt possesses only a very thin cuticle. A fact that is also concerned here, on the other hand, is that with a real "pupal molt" of young ligated abdomens shown in Fig. 10, it arises from the development of a typical pupal abdomens within such pupal cases (see Fig. 9).



Fig. 10a

Fig. 10b

Fig. 10 The lattice structure of the plate (P) in 2 Day21 hr. old ligated abdomen that then pupated in a: after implantation six 1 Day21 hr. old ring glands, in b: after implantation of two Day 5 21 hr. old ring glands. 40/1.

Also the other internal organs at this time of dissection show clearly the initiation of pupation processes. In the case of the series N these are shown in Fig. 11-13.

In Fig. 11, first, the central intestine of a control larva dissected at the same time is shown on the left (a). Due to its thick larval musculature the central intestine in the transmission light appears obscure. A completely different appearance in comparison to it is the central intestine of the pseudopupa (b). Due to the decrease of the larval intestine musculature the central intestine here is transparent. On the inside we see therefore the old larval intestine epithelium conglomerated to the so-called "yellow body" at the same time (G). Furthermore also the larval stomach blind hoses disappeared.

The ovary of the same case had grown and showed a typical pear form (Fig. 12, b).

Finally also the brain exhibited the characteristic changes for the first day pupae. One pays attention to the crosswise oval form of the hemispheres (Fig. 13, b) in relation to the round still smaller hemispheres (Fig. 13, a) the control larva.

We do not see therefore that ring gland implants of the late second larvae stadium exercise a clear metamorphosis effect also in unligated larvae of the third instar,

that expresses itself here in a premature pupa, while a metamorphosis effect did recognizably flow in on the unligated third instar through the ring gland of the early second instar.

In a similar way as ring glands of the early second larva instar now also such of the first instar cause a premature pupation when implantated into unligated



Fig. 11. Midgut, in a: a control larva, in b: a pseudopupa from Series N.G = yellow body. 20/1.
Fig. 12. Ovaries belonging to Fig. 11. a = Ovary of the control larva; b = Pear overy of

pseudopupa. 50/1.

Fig. 13. Cerebral eyes complexes belonging to Fig. 11. a = controllarva: round hemispheres (H);b = pseudopupa: broadly oval hemispheres and strongly grown scalp area of the eyeantenna imaginal discs (A). 30/1.

larvae of the third instar (see Table 6, Series Q). In this case eight 21 hr. old brain-ring gland complexes were implanted¹) into the host larvae. In all 13 cases an acceleration of pupation occurred around at least 16 hours (compared to first pupated control). All of these puparia had to a large extent the normal form, so they are not shows with italic writing (for pseudopupae) in the table. The dissection was performed before the first pupation of the controls.

¹) In order to avoid an injury of the ring glands, often involved eye antenna discs adjoined with the brain ring gland complexes, as well as with the isolated ring glands were not removed,

		Table 6. Prema	ture R	eleas	e 01	h up	arium	E O I	E at	q uo	y Rin	6 BI	an d	of	Irs 1	Ins t.	ar Là	BUNG					
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	ring glands	ins tar)				-	_		_	_		- 62					2		-	-	-	- 0	
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	ring glands	ins tar)																		_	_		
C ontrol	4	Day 2 21 hr.			-		-	_			-								÷		-		
	Four	Day 3 21 hr.															-			-	-		
ð	21 hour	(first half of the																		_			0
	ring glands	third instar)		_	-		(M	10	_	_		- 22					1		-		-		
C ontrol	85	Day 3 21 hr.										-		60	10	55	26	54					27

In 12 of the 13 the development had reached a finished pupal body; only one case showed more near to the above described picture of "cryptocephalic pupa."

In other studies ring glands of the first larval instar as well as different aged ring glands of the second larva instar were transplanted in unligated larvae of the second stage.

Series O and P of Table 6 show first the results after implantation of ring glands and/or brain ring gland complexes of the first larva instar. In all cases it came to the formation of pseudopupae (indicated in the table by italic writing).

One mentions that with all these pseudopupae as with those of series R which be described below it can be described that the second larval molt preceded the formation of the puparia. This was recognized clearly by the form of the mouth hooks in the puparium.

Before pupation of the first control, the pseudopupae had worked through development to the "cryptocephalic pupa" at the moment dissection () in all cases of the series P and in 7 cases of the series O. One case in Series O had developed a finished pupal body (Fig. 14). That smaller pupal body of the pseudopupae importantly compares with that first pupated (Fig. 15).

Fig. 16 and 17 may at the same time bring to the series of O and P some examples of the introduction of histological changes that likewise come prematurely in the pseudopupae. In both cases it concerns cryptocephalic pupae.

It is recognized in Fig. 16a the development of Zapfen (= eye color pigment cells) (Z, see VOGT, 1942b) in the scalp regions of the eye antenna imaginal discs, in Fig. 16b form of the yellow body (G) discharged in the larval intestine epithelium, and in Fig. 16c the first unit of the ovariole within the pear-shaped ovary.

Fig. 17 shows furthermore the crosswise oval form as well as the absence of ommatidial cluster formation in a brain hemisphere the pseudopupae of Series O (see a contrast to it in the larval hemisphere in Fig. 24!).

Also the histological analysis of the implanted ring gland as well as the host ring gland resulted in interesting findings. First the host ring glands of the pseudopupae showed a vacuolarization (Fig. 18), as is normally to be found in about 16 hour into the pupa stage. Since at at the time of dissection still no control larvae had pupated itself, thus cycle of the host ring gland was changed also under the secretions that flowed from the young ring gland implants. However, on the other hand, the implanted ring glands likewise showed its own development deviating from the norm. Thus 2-3 days after the implantation the large thigh cells (R) clearly exceeded the size which they would have reached at the same time in situ (see Fig. 19 and 20 with Fig. 21!). In contrast to this the cells of the central part (z) had hardly continued to develop (one compares oneself, e.g., the closely situated three nuclei left in the central part (z) in Fig. 19 with the nuclei in Fig. 21 lying far apart due to the larger cytoplasm!) Thus the large thighs cells showed an accelerated growth and the

central cells showed a restrained growth.



Fig. 14

Fig. 14. From the pupal case of prepared pupal of a pseudopupa of the series O, six hours before pupating of first control. 10/1. Fig. 15. Normal pupal body from the control series O. 10/1.

bodies



Fig. 15





Fig. 16. Organs of a crytocephalic pseudopupa the P series. In A: Zapfen (= eye color pigment cells) (z) the scalp of the uneverted eye antenna imaginal disc; in b: yellow body formation (G) in the midgut; in C: first ovariole imaginal disc. A: 500/1. b: 100/1. C: 600/1.

Fig. 17. Hemisphere of a pseudopupa of Series O. One pays attention to the crosswise oval form and the absence of the ommatidial cluster formation. 200/1.







Fig.21a



Fig. 19



Fig. 21b







Fig. 21c

Fig. 18. Vacuolization in the host ring gland of a pseudopupa (Series P). R = thigh cells, V = vaculoes, z = central part. 600/1.

Fig. 19. Implanted ring gland of a 21 hr. old donor larva 56 hours after implantation into a 2 Day21 hr. old larva. Closely situated nuclei in the central part (z). Hypertrophie of the large thigh cells (*R*); see Fig. 21. 500/1

Fig. 20. Similar case as Fig. 19, 70 hours after implantation. Very closely situated nuclei in the central part (z), R = thigh cells. 500/l.

Fig. 21. Three cut sections of a ring gland of a 3 Day 15 hr. old larva. Ao = Aorta. C.c. = corpora cardiaca. R = thigh cells. z =central part. 500/1.

Table 7 brings the results after implantation of different age ring glands of the second instar into unligated second instar larvae.

First, we see that implantation into late second instar larvae with ring glands of the early second stage clearly prematurely releases puparium formation (series of R), as we showed it above with implantation into third stage larvae (Table 5, Series N). It developed also here again because of the premature induction of the pupating processes the abnormally formed puparia strongly characteristic of pseudopupae (suggested in the table by italic writing). About 20 hours after the occurance of the first outside pupating features the internal organs also showed typical metamorphosis transformations with simultaneous formation of a cryptocephalic pupal body.

In Fig. 22 such is shown as prepared from the pupal case of a crytocephalic pupa. On the left body side the everted leg discs (B) and wing discs (F) are seen.

As the further example of the premature introduction of metamorphosis changes, it would be standard to assess the brain. Figure 23 shows dissected clearly the longer wide diameter and there being missing the development of the ommatidial cluster (\ddot{a} . B. and i. B.) existing out of embryonic cells in comparison with the brain the same time control larvae. (Fig. 24).

Added it is still that the brain hemispheres of the ring gland donors of the R series were implanted in each case into other host larvae (S series). We, like in former times in the Series E Table l, here also see an inefficacy of the brain implants.

On the other hand if ring glands of the late second stage were implanted into host larvae of the early second stage (Table. 7, Series T), then the hosts showed premature puparium formation in 10 % of the cases. The remaining 90 % pupated themselves about at the same time as controls. The ring glands of the late second stage cause thus usually no acceleration of puparium formation. The 10% that were exceptions might be best explained as due to self-development of the implants. These are the host ring gland approximately 24 hours ahead in their development ahead and would correspondingly become "pupation ripe" earlier in normal development.

Finally, Series U shows that also ring glands of the early second stadium do not release premature pupation when implanted into host larvae of the same age. An explanation for the very effective metamorphosis effect in all the above studies with ring glands of this stage, but which is missing here, will only be attempted in the Discussion. Excluding the last trial series, we are taught that with transplantations into unligated host second and third instar larvae, that

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		24 24000	Series	number		œ	S	Control	т	∍	Control

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Fig. 22a



Fig. 22b



Fig. 24

Fig. 22. Prepared from the puparium of cryptocephalic pupae of Series R, everted leg (*B*) and wing disks (*F*) eight hours before pupation of the first control. a: 10/1; b: 21/1

Fig. 23. Hemisphere of a cryptocephalic pupa (Series R) eighteen hours before pupation of the first control. One pays attention to the crosswise oval form and the absence of the ommatidical cluster formation compared with Fig. 24 wherein is shown the hemisphere of a control larva dissected at the same time. 200/l.

Fig. 24. Larval hemisphere with ommatidical cluster formation, see Fig. 23! \ddot{a} . B. = outside, *i*. B. = internal ommatidical cluster formation . 200/1.

after implantation from ring glands of the first and early second instar larvae, premature pupation of the host animals clearly takes place, while the same is missing after implantation of ring glands of the late second instar.

III. Promotion by implanted ring glands of developmental processes playing out during larval development

We turn now to the third initially question posed: Can development process, which normally play themselves out within the larva stage, be promoted likewise by implanted ring glands?

For the solution of this question the influence of the larval development of young eye antennal imaginal discs as well as young testicle were examined. In particular these two organs were selected because during the larva development there are already clearly exhibited structural changes, apart from strong growth in size, that indicate the beginning of "internal" metamorphosis.

a. Findings on the eye antenna imaginal discs

I. Dissection results after pupation of host abdomens has occurred

We begun with the results for young eye antenna imaginal discs. To assist in understanding the results, first the normal development of these imaginal discs must be described briefly.



Fig. 25

Fig. 25. Six eye antenna disks of 2 Day0 hr. old larvae of the second instar. 40/1.



Fig. 26



During first larval stadium and the first half of the second larval stadium the eye and antenna imaginal discs form a common approximately round disc (Fig. 25). Toward the end of the second larva stadium the antennal imaginal disc stands out, due to a stronger width growth of the eye disc (Fig. 26), which at this time is still a completely flat disc. During the third larva stadium the first bulge-like foldings in the eye disc develop (Fig. 27). Somewhat later an elliptical folding appears inside the antennal imaginal disc, which is the actual antennal system (Fig. 27, b). At the conclusion of the "prepupal" period (i.e. briefly before the puparium formation) there is an important growth in the width diameter of the eye imaginal disc as well as the antennal imaginal disc (Fig. 28).

We can divide thus the "larval" development of the eye antennal disc into three stages: Stage 1 = stage of the round disc; Stage 2 = beginning of outside separation between eye and antenna discs; Stage 3 = bulge stage. Here the Stage 1 covers the early larval disc development to including the first half of the second

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larva stadium, Stage 2 corresponds the second half second larval stadium and Stage 3 the third larva stadium. This organization was used for the classification of the eye antenna imaginal discs all series of tests.

The two eye antenna imaginal discs of a young larva were implanted alone (= control series) or together with ring glands of different stages into isolated abdomens 4 Day 21 hr of old donor larvae, after it was first determined that only in a few cases will they develop themselves further when only implanted.









Fig. 28. "Prepupal" eye antennal imaginal discs present at the formation of the puparium. The wide diameter of the eye area reaches three times as wide as high (See Klammer). 40/1.

Furthermore 4 Day 21 hr. old eye antennas disc implants were used to compare against.

The young eye antennal discs were always transplanted after careful preparation of the ring glands with the entire central nervous system (hemispheres + suboesophagealzapfen) of the donor larva, in order to facilitate in this way the finding of the implant. Since this was done with both the controls and with the ring gland series, the observed differences between control and cases with ring gland must be due to the hormones released by the ring gland.

Due to the small size of the organs, the often during the pulling out of the eye antennal imaginal discs by the mouth hooks, the neighboring antenna area gets injured (see e.g. Fig. 29, C and Fig. 38 j^2 !). Therefore, for classification, the stage reached by the eye implants was decisive. For the two eye antennal imaginal discs, in each case only the better developed was scored.

In a first series of tests young brain-eye-antennal complexes (abbreviated in the following with GAK) were transplanted from 1 Day21 hr. of old donor larvae

(= early second larval stadium) together with six ring glands, likewise from 1 Day21 hr. donors. The results of this series were already described briefly in a provisional report (1943). They have been increased around the cases of a further daily study and are shown here (Table 8).

Table 8. Promotion of larval development, by implanted ring glands, of early second larval stadium young eye antenna systems, in isolated abdomens. All abdomens with implanted ring glands had formed a puparium 1-2 days before the dissection.

Series number	Number and age of ring glands	Develop Eye A	omental Si Young .ntennal Im	tage of nplant	Stages 2+3	Stage 3	n
	implanted	1	2	3	111 70	111 70	
V	Six Day 1 21 hr. old ring glands	-	-	35 ¹	100.0	100.0	35
Control	-	49	37	3	44.9	3.4	89

As the table shows, 3-4 days after the implantation the young eye antennal discs of the ring glands series had themselves in 33 cases begun developing up to the Stage 3 and in two further cases up to the cap stage. In contrast to this result, at the same time 49 of the young eye antenna discs implanted alone (control series) were still at Stage 1, 37 at Stage 2 and 3 at Stage 3. These results thus showed that only 42 % of controls reached Stage 2 and 3% reached Stage 3, while the 100% of the implants of the ring gland series reached at least Stage 3. Also, the ring gland implants promoted those development processes in young eye antennal discs which normally take place more during the second half of the second larval stadium and the third larva stadium.

Here 1-2 days before dissection of the ring gland series all abdomens had themselves pupated, older eye antennal discs transplanted at the same time with the young GAK were always on the cap stage (see e.g. VOGT, 1943, Fig. 1). As I already stressed in the provisional report, the young eye antennal discs, apart from two exceptions, were thus still at a larval development stage, when the older implants had aleady without exception gone through the first pupating changes. Thus the younger imaginal disc units reacted to the ring gland hormones in a manner contrary to the older units, in that the younger units first went through a larval advancement. If these younger units are sufficiently advanced, then they can also react further also with typical pupation changes. This was seen, e.g., in the two cases already mentioned above of a beginning cap step, as well as further ______

¹) 2 of the implants were already beginning the cap stage.

cases of cap step that I recovered in a similar trial arrangement in which I dissected the isolated abdomens first 5 days after the operation.

It begs the question now whether the development of the implanted young eye antennal discs just described must be called quite normal, i.e., whether it would have led to a complete adult differentiation if you had granted the experiment an accordingly long life span. This question had to force itself upon all the more, as BODENSTEIN (1939a) found that that when young eye antenna discs are implanted into unligated mature larvae they cannot differentiate themselves to the adult stage.

Therefore three days after implantion into the isolated abdomen some of the young eye antennal discs were taken from these and transplanted again, this time larvae into unligated larvae. All implants differentiated themselves totally.

From these findings it turns out that the advancement of the young eye antennal discs observed in the ligated abdomens is quite normal.

This result seemed to stand now however in contradiction with above the findings of BODENSTEIN already mentioned. Either being missing the differention to the adult in the studies of BODENSTEIN had to be explained in another way, or the reaction of young eye antenna discs found above applies only to the special conditions of the isolated abdomens.

To clarify this question, in repeating the studies of BODENSTEIN, I examined first whether the initial development of young eye antennal discs implanted into unligated mature larvae is identical to that which takes place with implantation in isolated abdomens.

For this purpose, GAK taken at the early second stadium¹) and/or at the moment of the first molt were dissected two days after their implantation into mature larvae (for these and all following investigations I used only the host larvae which themselves had pupated within the first 20 hours after the implantation). Now also here the young eye antennal implants had gone through a typically larval advancement during the host's first pupal day - in addition, only such larval advancement was seen, so that without exception they had reached the stage 3 of our above organization upon the dissection on the second day of the host quiescent pupal stage (e. g. 20 cases with implantation

¹) only when taken toward end of the second larval stadium for implanation into mature larvae would implanted eye antenna disc show for the first time an imaginal differentiation (see e.g. Fig. 36).

of GAK, more of which had been taken at the first molt than early in the second stadium). This applied in the same way to implants from host larvae that had already pupated shortly after the implantation operation, which at the moment of dissection were thus already at the end of the second day of the quiescent pupal stage. This purely larval development entered also the presumptiven scalp area of the eye antenna discs: while the eye antennal discs of the host formed pupal head cuticle already often 24 hours before, the implants still did miss reaching formation of pupal head cuticle.¹)



Fig. 29

Fig. 29. Eye antennal discs from a 1 Day21 hr. old larvae in a: two days after implantation into mature larvae; in c: two days after implantation into 2 Day21 hr. old larvae; in b: Eye antennal disc of the host larvae belonging to c.



Fig. 30



Fig. 31

Fig. 30. Eye antenna discs taken at the moment of the first molt of 1 Day13 hr. old donor larvae in a: after isolated implantation into a mature larva; in b: after implantation as brain eye complex into a mature larva; in c: after implantation as brain eye complex into a 3 Day

21 hr. old larva. All implants two days after their transplantation. 40/1.

Fig. 31. Eye antenna system of a 1 Day13 hr. old donor larva three days after implantation in a 2 Day 21 hr. old larva. Increase of the widest diameter in the eye part. 40/1.

¹) this stands contrary to the behavior well known to us otherwise since the investigations PIEPHO (1938) on implanted pieces of lepidopteran cuticle. If e.g. the latter was taken even from the embryo and transplanted into mature caterpillers, then they pupated themselves under jumping over all larval molts synchronously with the host. This contrast could be based on the fact that into the cuticle pieces transplanted by PIEPHO the larval (not solely the adult as with *Drosophila*) hypodermis cells separates from the pupal cuticle.

Figs. 29 and 30 bring some examples to such larval advancement of young eye antennal implants after there occurred pupation of the host.

In Fig. 29a we see three young eye antenna discs taken from 1 Day21 hr. old donor larvae two days after their implantation into mature larvae. In comparison are shown in c two eye antenna discs of equivalent donor larvae, which were at the same time transplanted into Day 2 21 hr old unligated larvae (thus compared with the donors only around 24 hours older). Finally, with b are represented the eyes belonging to the host larvae of c. The implants shown in a are still typically larval in character, as we find naturally in the implants of row c. They (in Fig. 29a) differ (from Fig. 29c) only by the width of the eye part already being larger in the comparison to the height, indicating the beginnings of "prepupal" growth (see Fig. 28!).

Fig. 30 shows corresponding for eye antenna discs corresponding to a and b, which at the moment of the donor (first) molt were taken from the donor larvae and transplanted into mature larvae. (Fig. 30a displays the implanted young eye antennal discs that were isolated without the brain complex, showing at the same time the lack of contribution observed here of the brain to the behavior of the eye antenna discs). In a and b again in the comparison to the height is already the larger width diameter of the eye part, and one sees in contrast to that the eye antenna system for treatment c, which are those that became implanted into only 3 Day 21 hr. old (i.e., not "mature") host larva. Such transplants of young eye antennal discs into larvae yet to be mature do not show an appropriate relative increase in breadth of the eye area in only about 24 hours after the implantation (Fig. 31).

A typical larval young implanted eye antennal imaginal discs, but with advancement accelerated at the same time, is thus found during the first and second day of the quiescent pupal stage of the host

These results prompted afterwards a further pursuit of the development of the young implants within the host pupae.

In dissected host animals, the first three days after the implantation, all young eye antennal discs were now at the cap stage; at the same time they possessed a pupal cuticle with their characteristic still silvery appearing "pupal molt pigment" in the head region. The pupal molt of the implant thus takes place after only about 11/2 day of the pupal molt of the host!

This findings have still another interesting aspect. The implant accomplished a pupal molt at a time when the host animal the developed structures were characteristic of the so-called "imaginal" molt (e.g., formation of hair). I received the same result with implantation of larval eye antennal discs into pupae. For example, when I implanted eye antennal discs from 3 Day 21 hr old into 65 hr. old pupae, those implants then remained larval. On the other hand, if I transferred the discs into pupae which 52 hours before the operation had formed a puparium, and had thus 38 hours before the operation gone through a pupal molt, then the implants (21 cases) showed without exception that at the time of their host's eclosion to the adult, the implanted discs show the caps-yellow or pale yellow pigment stage with a typical pupal cuticle with its own gold-yellow pupal cuticle pupal cuticle pigment.

such implants (inside a host that was stimulated to molt to the adult) received at that moment of transplantation to stimulus for a molt to a pupa, thus at least not only releasing active substances for the "imaginal" must be present in the host's blood at the beginning of the third day of the quiescent pupal stage, but also still such for the "pupal" molt, if one wants to accept with the majority of the authors that the active substances for the pupal molt and the adult molt are unequal active substances. In this case the hypodermis of the host animal and the implant would have to react to both groups of hormones in each case due to a different sensitivity. With the acceptance of a "different sensitivity" it becomes necessary to accept for the findings (raised here with *Drosophila*) of a group of active substances. With this hypothesis the hypodermal cells react to the same group of hormones with the developmental stage that follows directly from their presently ripe condition. Only future investigations will bring only a decision between the two views.

We return now to the description of the test results interrupted above!

Up to eclosion of the adult host the implants had still somewhat continued to develop. In all 17 examined cases now the pupal cuticle pigment showed a strong gold-yellow color. Beside 7 colorless cap stages, furthermore in 10 of the cases yellow eye pigment had developed in the eye plate. During the histological investigation such eye plates showed the stage 4. In no case had the disc come to adult differentiation.



Fig. 32. Degeneration of eye antennal disc implants from 1 Day13 hr. old donor larvae that were developed up to stage 4 after after implantation into a mature larva. Dissected on the day after eclosion of the adult. 500/1

Also, adult differentiation was not found in 19 cases that were dissected by the first five days after the emergence of the adult. Instead of its dying the implant appeared to have already went into action. The yellow eye pigment had discoloured only to a dirtily ocher-brown pigment and the ommatidia here showed in the histological preparation of degeneration features, so that the round ommatidia characteristic of the stage 4 could hardly be recognized anymore (Fig. 32).

In summary it can be stated thus that the initial development of young eye antennal discs upon implantation in mature unligated larvae quite resembles that observed in the isolated abdomens, however further advancement is then missing and gradually a dying occurs.

On what are these disturbances of the advancement based? For clarifying this question the young eye antenna disks two days after their first implantation into unligated mature larvae were transferred again also here into unligated mature larvae.

Again the reimplanted disc differentiated themselves out in all 20 cases¹) in which the host animals got over the operation and eclosed.

According to the small size of the eye antenna discs at the moment of the second implantation (compare e.g. Fig. 30, a and b with the eye antennal discs of older larvae, Fig. 27, b) they formed only small differentiated eyes. The individual ommatidia showed thereby a completely normal structure, and was only somewhat larger than the appropriate







Fig. 34a

Fig. 33. Second eye, to an eye of the same donor larva shown in Fig. 30, after reimplantation into a mature larva. Dissected shortly after eclosion of the adult host. 500/1.



Fig. 34b

Fig. 34. Second eye, to an eye of the same donor larva shown in Fig. 30 in b (left) after reimplantation into mature larva. Dissected shortly after eclosion of the adult host. In a: Eye region 600/1; in b: Antenna with arista (*Ar*). 200/1.

ommatidia of eye antenna implants which were taken from mature larvae on the same days and transplanted into mature larvae. (one compares e.g. the lens size of the latter in Fig. 35b with that the former in Fig. 33 and 34a!)

These findings might be partially based on the already normally larger cells of young imaginal discs. Thus we received similar findings also with the single time

¹) into 14 of these cases the eye implants before second transplantation were separated from the brain and implanted alone. The implants with and without brain differentiated themselves in the same way.

implantation of 2 Day 21 hr. old eye antenna systems into mature larvae (Fig. 36 b). Parallel with the small size of our for the second time injected eye implants, therefore only a smaller number of ommatidia are formed, as is already well know for young-in-old implantations (see Fig. 35a and 36a!).

By the way, the adult differentitation of for-the-second-time-transplanted-implants applies in the same way to the antennal area, as Fig. 34b shows. Clearly recognized is the coloring of the arista (Ar) and the smaller antennal hair.









Fig. 36. Eye antenna implant of a 2 Day21 hr. old donor larva taken at the moment of the second molt and into implanted into a mature larva. A,: 100/ 1, b: 500/1

Furthermore it is still emphasized that for the implants show in Fig. 33 and 34 there is shown one eye each from the same donor larvae for which their respective other eye is shown in Fig. 30 in a and/or b (left) photographed after the withdrawal from the first host pupa. They were thus at that moment of the second implantation in the represented developmental forms in Fig. 30.

Therefore also these findings show a quite normal initial development of the young eye antenna discs in the unligated hosts

For the differentiation to be omitted for the single time implantation conditions must therefore have another reason. Here now the following explanation seems to me as the most probable. It was already mention on page 399 and also by some examples shown that hormonally driven differentiation processes for adult development are promoted by ring glands. It therefore seems reasonable to assume that, in above case, toward end of the quiescent pupal stage the hormone concentration in the host blood is not sufficient any longer for the completion of the adult differentiation of the young implants. Speaking in favor of this explanation is the fact that such young eye antennal discs adult-differentiated themselves if they were thus transplanted two days after the operation again into a mature larva, which is an environment rich in metamorphosis hormones. The conclusive force of this finding is strenghtened by a similar determination by me in previous publications (1941 and 1942a). We have an example, in the degeneration due to lack of active substance that results from a homoplastic (same species) transplantation, of the just given explanation of the dying out of the only once implanted eye antennal discs. I described a similar dying already in a previous publication following heteroplastic (different species) transplantions of eye antenna discs and ovaries. I also accept here a lack of active substance as a principal reason of the implanted disc dying, since I could prevent it to a large extent by simultaneous implantation of donor-species-characteristic brain ring gland complexes.

The differentiation observed above after reimplantation speaks at the same time against acceptance of BODENSTEIN (1939b), according to whom only the presence of the thoracic tracheal center is necessary for adult differentiation (the "pupal differentiation center" of BODENSTEIN). In that model, it would be incomprehensible why the young implants [into unligated hosts] probably reached the cap stage and often also an early pigment stage with one time implantation, but itself despite the presence of the thoracic tracheal system did not finish differentiation of itself¹), while they were able to do this during a new transplantation into a new environment.

2. Dissection results with host abdomens in which pupation was omitted

In Section I (Series V) the promotion of the larval development of young eye antenna discs took place via implanted ring glands in isolated abdomens,

¹) Supplement with the amendation: This applies in still higher measure to experiments accomplished subsequently. In these I received also a further differentiation of the young eye implants by additional implantation of three pupation-mature ring glands 24-28 hours in situ after the pupating of the host animals. I bring a representation in more detail in another place.

which went through at the same time pupation changes. It was tried therefore in two further series to achieve a similar development promotion also in such abdomens which were still in larval, because this is nevertheless the condition which corresponds to those in normal larval development.

It was thus performed in such a way as to arrange that quantity of the pupation hormones delivered by the implantated ring glands remained less than maximum. This was accomplished in two different ways.

In Series (W) 4 Day 21 hr old ring glands were used as a source of hormone, because according to experience three days after implantation of ring glands of this age only cause about 40% of the abdomens to undergo pupating processes, thus leaving approximately 60% abdomens remaining counted as unpupated (See Table 3, Series H and Table 4, Series L).

In the other Series of studies (X), the two free thigh ends (See e.g. S in Fig. 51) that were Day 5 21 hr. old (thus almost pupate-mature) were used as implanted ring glands. A sufficient number of abdomens here also still remain unpupated.

Table 9. Promotion of larval development by eye antennal discs taken at the first molt that were
implantated into isolated abdomens. The host abdomen at the time of dissection (3 days after the
operation) was still in a larval condition.

Series Designation	Number and Age of Implanted Ping Glands	Develop Ey	omental Stage /e Antennal Im	of Younger pplant	Stage 2+3 in %	Stage 3 in %	n
	King Olahus	1	2	3	111 70	111 70	
W	One Day 4 21 hour ring glands	13	4	21	65.8	55.3	38
Control	-	65	6	-	8.5	0	71
х	One Day 5 21 hour Thigh Ends	14	7	21	66.7	50	42
Control	-	68	8	-	10.5	0	76

The results of both series are summarized in Table 9. In the case of each series, the only test discs scored were those in which the host abdomens were still larval at the time of dissection. The control series shows, first, that those young brain-eye-antennal systems (GAK) taken at the first larval molt for implantation reached Stage 2 in our organization of eye development in approximately 10% of the cases and in no case reached Stage 3. Different on the other hand were the young eye antenna discs in the ring gland series. Here 50 to 55% showed Stage 3 and beyond that still another 10-15% showed Stage 2.

Some characteristic examples are on the basis shown in the Figs. 37-40.

The Fig. 37 and 38 shows young eye antenna implants of Series W, the three labelled j and/or j2, taken three days after the implantation had reached Stage 3 (= bulge stage),





Fig. 38

Fig. 37 and 38. Eye antennal discs three days after implantation in isolated abdomens. a = older [4 Day 21 hr.], j = younger eye [at first molt] antennal discs with simultaneous implantation of 4 Day 21 hr old ring gland. $a^k = older$, $j^k = younger eye$ antenna discs of the control series.

Fig. 39 and 40. Eye antennal discs three days after implantation in isolated abdomens. a = older, j = younger discs when simultaneously transplanted with two thigh pieces from Day 5 21 hr. old ring gland. a^k , $j^k =$ control implants. 40/1



Fig. 39



Fig. 40

The largest (for the same days) of the dissected young control implants are labelled with j^k and/or j^{k1} and j^{k2} . We see j^{k2} falls here into Stage 2 of our organization.

Three other cases (Series of X) are shown in Fig. 39 and 40 (j, j^1 and j^2). j^2 corresponds to a younger, and j and j^1 to an older, bulge stage. From controls (j^k) in Fig. 40, only j^k has reached Stage 2.

In Fig. 41a, a case from one is finally represented that is not shown in the Table series, in which the [5 Day 21 hr.] ring gland transverse arch in its whole (Fig. 51 above right) was also implanted, staying after separation from the free thigh ends. The eye antennal disc implant (j) is here also at Stage 3.

Furthermore in all illustrations were portrayed the older [4 Day 21 hr.] eye antennal discs that were implanted simultaneously young GAK. The eye antennal discs of the ring gland series (a, a^1 and a^2) differ also here clearly by their size of those of the control series (a^k). While in Fig. 37 and 41 this growth can be interpreted as already prepupal, Figs. 38 and 40 concern a still purely larval growth, which above all is clearly shown by the still typical elliptical form of the antennal imaginal disc (see Fig. 38 <---).



Fig. 41



Fig. 41. Eye antennal discs three days after implantation in isolated abdomens. a = older, j = younger disc with simultaneous implantation of the transverse arch of a Day 5 21 hr. old ring gland. a^k , $j^k =$ implants of the control series. 40/1.

Fig. 42. Eye antennal disc of Series W after reimplantation into one mature larva. Dissected shortly after eclosion of the adult. The disc unit had been transplanted together with a 4 Day 21 hr an old ring gland into an isolated abdomen and in the latter had developed itself three days further. 500/1.

We see thus that ring gland implants already release growth and shaping processes in young eye antennal discs, even if the quantities of pupating hormones delivered by them are not sufficient yet to release typical pupation changes in competent host organs as well as in the older eye antenna disc.

We are entitled to interpret here that the development processes experimentally induced in the young eye antennas-implants, being normal, show among other things the fact that these implants differentiated themselves with their reimplanation, (this in unligated larvae), completely normally. Fig. 42 shows this for a reimplanted eye antennal disc of Series W.

In contrast to this, Fig. 43 brings an implant brings of the control series, which was at the time of the second transplantation still at Stage 1. In accordance with expectation, it developed only up to the light yellow pigment stage (Stage 4).



Fig. 43

Fig. 43. Eye antenna disc of the control series after reimplantation into a mature larva. Dissection was performed shortly after eclosion of the adult. The Stage 4 disc unit had been previously transplanted alone into an isolated abdomen and after three days was still at Stage 1. 500/1.

b. Findings on Testicles

I turn into now to the second organ, i.e. the testicle, that I examined. Because during the entire larval development this experienced a substantial size increase while under substantial preservation of its exernal form (see rows of b of the Fig. 47 -- 49), I was predominantly limited to the measurement of the size. The experimental assembly resembled that used in section the IIIa (P. 419) with the only difference being that in place of the young eye antenna discs, the discs of young testicles were implanted here.

In a first series of tests (a) testicles of the first larval instar (that is, 21 hr. old larvae became donors) together with 4 Day 21 hr old eye antennal discs partly alone, or partly with a 5 Tg. 21 hr. old ring gland, were implanted into isolated 4 Day 21 hr old larval abdomens. (To facilitate regaining the testicles, at this stage the testicles with a large part of the fat body around it were transplanted). Three days after the operation the abdomens were dissected and the testicle implants were measured. The results are graphically presented in Fig. 44.

Only implants in abdomens that pupated were considered here and also in all following series of this section, since the occurrence of a puparium indicated the technical success of implantation of the ring gland. In this way there could be eliminated at least one the variability factors causing very increased dispersion caused by on very variability-increasing factors.

The shifted curve of the Fig. 44 represents the implants of the ring gland series. It is clearly moved to the right in relation to the control curve. This misalignment is statistically secure (difference of the average values $D = 10.5 \text{ X m}_{\text{Diff}}$). A clear growth of the young testicle implants thus takes place under the influence

influence of the implanted ring gland. (Also compare the three representative implants in Fig. 47 row a with the testicle implants of the control series illustrated in row b!)

Apart from the outwardly recognizable size increase the testicle goes through, there are also histologically provable structural changes, which express themselves in the progressing spermiogenesis during the normal larval development. While the testicles of the control series always only contained spermatogonia (Fig. 50b), the enlarged testicles of the ring gland series already predominantly showed spermatozoa (Fig. 50a, when same enlargement is made). This is a developmental gradation, which for example the testicles reach in normal development at the beginning of the third larva stage.

Thus the ring gland hormones release development processes, which normally take place during the second larval stadium not only in the eye antenna discs, but also in the testicles.

Appendix. Supplemental remarks about the hormonal control testicle development

To my knowledge, such hormonal control of testicle development has not been described with insects. Therefore some test series are here briefly described, which supplement the trends of the findings just discussed.

The promotion of the testicle development by implanted ring glands, determined in section b, referred to the early larval testicle development. It can be proven in the same way also for the early pupale development. Thus the host testicles showed a clear enlargement in all isolated abdomens, which had pupated themselves under the influence of implanted ring glands of the most different age, if the abdomens survived at least one day beyond the first exhibition of the external pupation appearance.

In Fig. 46, as an example, a series of tests was picked out, in which a Day 5 21 hr. old ring gland was implanted into isolated 4 Day 21 hr old larval abdomens. Despite the small number of measured testicles, already here the size difference compared against the control testicles is to a large extent statistically secured (Diff = $13 \times m_{Diff}$).

If the abdomens showed "imaginal differentiations", then a particularly strong growth increase of the testicles went in parallel to these. (In these cases it varied in these cases of the length diameters between 40-45 micrometer units, see with the limit value in Fig. 46!)

p. 432



Fig. 44. Length diameter of the testicles of 21 hr. old donor larvae at the first Stage, 3 days after implantation in isolated abdomens. Broken line curve: control series only testicle implanted. Shifted curve: Testicle with simultaneous implantation of Day 5. 21 hr. old ring gland. [for data on 23 cases of transverse arches implants see pg. 435]



Fig. 45. Length of diameter of the testicles of 2 Day21 hr. of old donor larvae, which was taken at the moment of the second molt was taken and implanted to isolated [4 Day 21 hr.; see page 435] abdomens. Measurement 3 days after the implantation. Broken line curve: Testicle of the control series. Shifted curve: Testicle with simultaneous implantation of four thigh pieces of Day 5 21 hr. old ring gland.



Fig. 46. Diamter length of (host's) testicle 4 Day 21 hr. of old larvae, 8 days after isolation of their abdomens. Broken line curve: Testicle of controls. Shifted curve: Testicle after implantation of one Day 5 21 hr. old ring gland.

In these testicles were also more frequently seed thread (see page 401). Thus progression of the spermiogenesis was connected also with pupal testicle growth.







Fig. 47 through Fig. 49. Growth of 21 hr. (Fig. 47), 2 Day 21 hr. (Fig. 48), and 4 Day 21 hr. (Fig. 49) old testicle in isolated abdomens with simultaneous implantation of ring glands. These are cases from the Fig. 44 - 46 graphically displayed picture series. a = testicle of the test series, b = testicle of the control series. 40/1.

Furthermore the hormonal control of the testicle development with *Drosophila*, uncovered above, is interesting in connection with the following negative findings of other authors. After elimination of the imaginal corpus allatum no interference of the spermiogenesis was observed by THOMSEN (1942) in *Calliphora* and WIGGLESWORTH (1936) in *Rhodnius*. My own investigations at *Drosophila* pointed likewise to the absence of stronger interrelations between the imaginal corpus allatum and the testicle (1942a, see page 450). It therefore appears more probable that the promotion of testicle development received (see above) with implantation of whole ring glands would be due to the hormones of the big ring gland cells not on the active substances of the central ring gland part (= corpus allatum).

In order to examine this, in a further series of tests it was tried to switch off the hormones of the central ring gland part by exclusive implantation of the free thigh ends of the ring glands (Fig. 51, page 436).

In the purely technical way it must be noted that it is not intelligently possible to exclude in this manner with absolute security the occasional transmission of single corpus allatum cells. Nevertheless I believe to have reached probably reached this clearly by putting a cutting force beneath the transverse arch. On that I will still return in the discussion on page 442.



Fig.50

Fig. 50. Cut sections from two testicles of 21 hr. old donor larvae 3 days after implantation in isolated abdomens, in a: with simultaneous implantation of one Day 5 21 hr. old ring gland, in b: implanted alone. 500/1.

In the series b implants, in each case the two thigh ends of two Day 5 21 hr old ring glands were placed in isolated 4 Day 21 hr old larva abdomens with 4 Day 21 hr. old eye antennal discs and one testicle taken from the moment of the second molt. In Fig. 45 is shown graphically the results of the b series. Seen here is a statistically certain enlargement of the testicle under the influence of the thigh ends ($D = 19.6 \text{ X} \text{ m}_{\text{Diff}}$). In Fig. 48 three testicles (a) of this series as well as the three largest control testicles (b) of the same day study were live photographed.

On the other hand, the counter test of implanting alone the ring gland transverse arches showed that the active substances of the corpora cardiaca cells (Fig. 51, C.c.) lying in the thigh points are not necessary for the effect of the thighs pieces. The testicle implants showed a clear growth also here. The average value for 20 measured testicles amounted to 20.95 + 0.33 compared to the average of the control testicles of 13.65 + 0.20 (Diff = $18.7 \times m_{Diff}$.)

The just described results speak thus to a large extent for the assumption expressed above that it is the hormone of the large ring gland thigh cells which promotes testicle development.



Fig. 51

Fig. 51. Live photographs of two glands of Day 5 hour 21 old larvae. On left a whole ring gland, on the right the thigh ends (S) free from upper transverse arch . C. c = .Corpora cardiaca. 100/1

IV. The ring gland thigh cells as promoters of certain more larval development procedures and also of the puparium formation that is executed under metamorphic processes

This same degree of certainty which occurs as to control over larval testicle development by the large thigh cells of the ring gland can now be shown also for the larval changes of the eye antenna discs.

As already shown on page 428 (Series X), the larval development of the eye antenna discs is promoted by isolated thigh ends. Thus the hormones the corpus allatum cells for the observed promotion of the larval development of the eye antenna systems might not be of importance. The same can be concluded furthermore with still larger assurance for the active substances of the corpora cardiaca from the fact that young eye antenna discs likewise show an advancement after implantation of the ring gland transverse arch. For this 14 positive cases are put forth here (see e.g. Fig. 41), which were not mentioned in Section IIIa in a special table.

Since thus both with absence of the cells of the corpus allatum and the corpora cardiaca an advancement of the young eye antennas disks is promoted,

¹) Due to the large distance between the thigh points and the upper transverse arch the corpora cardiaca cells can be operationally switched off perfectly.

only the hormones of the ring gland thighs remain cells as promoters of the larval development of the eye antenna disc.

How does it stand now as to the production site of the hormone, with respect to release of puparium formation under the exercise of the procedures that normally regulate metamorphosis? For the solution of this question the behavior of the host abdomens can be consulted, and into these host abdomens of those series were implanted older eye antennal discs, and into which likewise were transplanted only isolated thigh ends and/or transverse arches. It begins with the findings from the implantation of isolated thighs pieces (Series X, page 428 and Series b, page 435).



Fig. 52. The widest-dimension diameters of (host's) testicle 4 Day 21 hr old larvae, 3 days after isolation of its abdomen. Left graph: Control testicle. Right graph: Testicle with simultaneous implantation of two and/or four thigh ends of 5 Tg. 21 hr old ring glands.

The abdomens of the series X and b had formed a typical puparium 2-3 days after the operation in 67 % (series of b) and/or 30 % (series of X). Although the pupated abdomens showed 2-3 days after the implantation already without exception first decomposition features of the fat body as indications of dying beginning, also in the internal organs typical pupating changes had occurred. Thus in the intestine the "yellow bodies" had been formed, and the fat body began to disintegrate. The host testicles showed a clear size growth (Fig. 52; the host's testicles of all abdomens which had formed a puparium at the moment of dissection were used here). The ovary was on the pear to ball stage and ovarioles had developed (Fig. 53a). A part of the 4 Day 21 hr old eye antenna discs implanted at the same time showed remarkable results. Although all abdomens had only formed a puparium during the second and/or third day after the operation, 18 of the 34 eye antennal implants already after 3 days were beginning the pigment stage.

In 9 of these eyes, the pigment development covered the total eye plate. Histologically the eyes had reached at least Stage 4 (Fig. 54); with the otherwise well beginning decomposition phenomena, it was not a concern therefor how one could suppose it. In a further abdomen, the implant had itself developed to Stage 5, and in a final abdomen to Stage 6 – (Fig. 55). Apart from the last case, the remaining abdomens showed the first beginning of a disintegration of the fat body and a first beginning or at least yet unshrunken yellow body.

The development of the eye antennal discs is thus here for instance around 11/2 days ahead of those of the other organs (cuticle, intestine and fat body). Thus it besides became also noticed that in comparison to earlier series of tests there was a remarkably high percentage (over 50%) of "imaginal differentiation".



Fig. 54. Eye antenna disc 3 days after implantation into an isolated abdomen along with the simultaneous transplantation of four thigh ends of Day 5 21 hr. old ring glands. The host abdomen pupated only after the second day after operation. The eye is on the yellow pigment stage (Stage 4). 500/1.

Fig. 55. Eye antennal implant, during same test arrangement as in Fig. 54, developed up to Stage 6 three days after implantation. The host abdomen pupated during the second day after the operation. 500/1.

Also the host abdomens, into which a ring gland transverse arch was implanted, formed typical puparia. In comparison with the abdomens receiving thigh pieces, the formation of the pupal case took place on the average somewhat earlier. In this case, of 23 abdomens that pupated, 10 had already formed a puparium after one day. The "internal" metamorphosis process was introduced also here in all cases. Contrary to the abdomens with thigh pieces however here never was a pigment stage. All abdomens showed likewise their first decomposition features after three days.

In summary we can thus say with the same certainty as on page 436 that from these findings it is confirmed that puparium formation is released by the large ring gland thigh cells.

D. Discussion

The collating of the findings presented here will to contribute to clarifying the following question: Do metamorphosis-promoting hormones already play a role with *Drosophila* in the normal larva development?

The results discussed in Section I informed that ring glands of the first and early second larva stage briefly released typical metamorphosis processes after their implantation in isolated abdomens of the third larva stadium. It was thus first proven that ring glands of the early larva stadia can form metamorphosis-promoting hormones.

Section IIa resulted in further a clear difference in the quantities of metamorphosis hormones produced by ring glands of different stadium soon after their implantation. Thus implanted ring glands up to and including the first half of the second larva stadium showed a clearly increased hormone delivery, which was reached and/or exceeded only again by ring glands toward end of the third larva stadium, in the comparison with ring glands of the late second and early third larva stadium. Since the implantations always took place in isolated larva abdomens of the same age, the ring glands of the different age donor larvae must have been, at the moment of the extirpation, in different functional conditions.

Is there a cycle in the normal larva development which coincides with the periods of increased hormone delivery of the implanted ring glands? This applies to a large extent to the molt. Thus we observe a decreased delivery of hormones by implanted ring glands of the late second and early third larva stage. It is now to be considered that at this time in normal development no molt is determined. On the other hand, the fact that we do not observe an appropriate developmental interval between the first and second molt might be based on the fast developmental sequence of these two molts. (Between the first and second molt is about 1.25 days, between the second larval molt and the pupal molt 3-4 days). This parallelism between the determining times of the molt and the observed

^{$\overline{1}$}) The second larval molt is determined in the late second larval stadium, see page 405.

increased delivery of metamorphosis-promoting hormones by the implanted ring glands provide initial experimental evidence that the metamorphosis-promoting hormones play a role during the release of the larval molt also with *Drosophila*. As I already mentioned on page 396, this is accepted by WIGGLESWORTH for *Rhodnius* and by FUKUDA for *Bombyx*.

In the series of studies of section IIb, ring glands of different age donor larvae were transplanted into unligated larvae of the second and third stadia. A clearly different effect resulted between ring glands first or early second and the late second larva stadium, despite expected disturbing reciprocal effects between the endocrine secretion system of the host and the implanted ring glands (see. e.g., page 412). The former [first or early second instar] clearly released a premature pupation of the host larvae. With the latter [late second instar] this was missing. Even if it cannot be decided here whether the premature pupation was released directly or only indirectly by the implanted ring glands, we nevertheless still have a difference in activity of the two groups of ring glands, as already resulted from the studies with the ligated abdomens. The conclusions, which had been suggested to us by those last studies, thus find further support here.

Still other findings communicated in section IIb are mentioned here. The otherwise strongly active ring glands of the early second stage did not release premature pupation upon their implantation into equivalent age host larvae. This can be explained by the obvious acceptance that a ring gland implanted into a equivalent host goes through and to no modification of the metamorphosis process, due to the time of their implantation with the ring gland of the host, the synchrony in their activity phase leads to also further a synchronous development. It is still pointed out that PLFUGFELDER (1940) made the same observations with *Dixippus morosus*. That author received only surplus larval molts with exchange of different aged corpora allata, while an exchange of corpora of the same age did not affect allata the number of the molts.

Apart from the findings of the sections I and II discussed so far, still another must be further emphasized, i.e., the remarkable outcome that implanted ring glands of the first and second larva stage caused a pupal molt and not a larval molt. This points out that we must accept there is an "inhibition factor" also with *Drosophila*, which changes the pupal molts induced by the young ring glands in favor of larval molts.

This inhibition factor obviously fails with implantation of young ring glands into different age hosts. In view of the relevant investigations of representatives of other groups of insects, the active substances of the corpus allatum represent this inhibition factor. If we regard with THOMSEN (1940) and DAY (1941) that the central ring gland part is the homolog of the corpus allatum other insects, and and we accept for it this uncovered function, then it must remain provisionally still unclear why the central part does not manifest this awarded determination of the larval molt so as to take over in normal development after its transplantation. Next is presented the acceptance provisionally, but not causally explained, that there is a reduction in the function of One can see a support of this view therein that the latter after central part. transplantation into older hosts stays in its development, as was shown on page 412. We do not need to derive therefore provisionally yet from the above findings that another organ determines the larval molt with Drosophila. Also BURTT (cited in WIGGLESWORTH, 1940) in still unpublished studies claims furthermore to have received the first experimental note for a restraining effect of the ring gland. Page 442 represents a second reference which can still be discussed on accelerated eye development after elimination of the central ring gland part.

Described in section IIIa is the promotion of "larval" development of young eye antenna discs by implanted ring glands. The development processes of the young eye antenna disks, which normally take place during the second half second and during the third larva stage, were released here by the hormones of the ring gland implants. That these induced development processes are to be interpreted as thoroughly normal shown by the imaginal differentiation of such young implants if, by repeat is transplantation, an opportunity for further development was given by more time in unligated host. The promotion of the larval development of the young eye antenna systems was also successfully released, even if the quantity of metamorphosis hormones delivered by the ring gland implants were not sufficient yet to cause pupating changes in the host abdomen or in the implanted older eye antennal discs (P. 427). We must accept that these conditions are particularly at hand for the first two thirds of the last larva stage. Therefore, it seems to me the permissible conclusion is that the ring gland hormones also promote in the normal development the "larval" development of the eye antenna disks. Transplantations of different ring gland sections (P. 436) probably furthermore made that we must see the ring gland thigh cells as the production site of hormones that promote the larval eye and antenna.

A second organ, whose "larval" development could be promoted by implanted ring glands was the testicle (IIIb). The results speak for it that its development is also driven by the ring gland thigh cells (P. 436). Here, for the first time, the hormonale promotion of the spermiogenesis that was uncovered with *Drosophila* is extended further to also the later development stages in the young pupae (S. 432).

Section IV of this work on transplantations of different parts of the ring glands brought similar note for the special meaning of the hormones of the ring gland to give egg cells a track to pupariation through metamorphosis processes. Here the remarkable findings were described that implanted 4 Day 21 hour eye antenna imaginal discs clearly showed accelerated development after simultaneous implantation of isolated thigh pieces, in comparison to the older host organs, so that three days after the implantation over 50% of the implants showed "imaginal differentiations." Since a similar behavior of the eye antenna discs was never observed so far by me in the other series of tests, one is tempted to attribute this accelerated development of the eye antenna discs to a specific sensitivity of the discs to the abolishment of the still hypothetical with Drosophila metamorphosisrestraining effect of the cells of the corpus allatum (= central ring gland part). At the same time is to be emphasized yet that this acceleration and the self-expressing special sensitivity therein does not concern the entire eye antenna disc, but rather the "scalp" areas are excluded from the acceleration. This recalls the proof by PIEPHO (1942) of the unequal reactivity of the different body organs and parts to alterations of the normal quantitative proportion of the hormones taking part in the control of metamorphosis.

At the same time I would like to look at the different behavior of the eye-antennal disc in response to the implantion of the thigh pieces versus the transverse arch as a support of the technical success in surgically removing the corpus allatum cells. It gives therefore more power of proof to the viability of the conclusion repeated as to the special meaning the thigh cells.

It still to be mentioned as of some interest that the differentiation processes in the ovary (ovariole formation, see page 437) operating during the quiescent pupal stage itself were likewise released by the thigh cells. Hereby apart from the meaning uncovered by THOMSEN (1940) for muscids it would arise then that the

corpus allatum cells have for the yolk development an independent meaning of the thigh cells for the differentiation processes of the ovary. It is not uninteresting therefore that in earlier studies I accomplished heteroplastic ovary transplantation between certain kinds of *Drosophila* [spp.] that pointed into similar direction. Thus the corpora allata of the receiving host hypertrophied concommitant with the formation of ripe eggs in the transplanted ovary, while the differentiation of the oocytes remained imperfect. Some such cases were already previously illustrated by me (1942a, Fig. 5b and C).

We come now to a last question. Are the hormones, which had a promoting effect on certain "larval" development processes, and which afterwards drove pupal metamorphosis changes, identical or not?

The controlling of the "larval" development of young testicle and young eye antenna discs could be achieved also by the thigh cells of nearly pupation-ripe ring glands. On the other hand, the thigh cells of the ring glands of the same stage also released puparium formation using metamorphosis processes. Therefore the most probable heuristic hypothesis appears to me that the same metamorphosis-promoting" hormones of the thigh cells are also those which drive the "larval" development processes in the organs mentioned above. This question can only be finally solved chemically. It [the heuristic hypothesis] encounters nevertheless a large difficulty in then accepting the existence of special "larval" promoting active substances versus active substances that promote the "actual metamorphosis." The development of many larval organs to the imaginal form is a gradual, which thus affects making a distinction of a "larval" stage from a "pupal" stage in which actual metamorphosis Spermiogenesis, for example, is a completely continuous process and also belongs. the development of the eye antenna discs shows nowhere a pause or a jump, which would justify a separation between larval and pupal development. One is therefore fairer to the natural conditions if one understands all of larval development as taking placing on the organs of the adult, so that "imaginipetal" structural changes are arranged as already the beginning of "internal" metamorphosis. Their controlling and those of the "actual" metamorphosis procedures by the same hormones¹) would actually lie closer with this view of the larval processes mentioned already.

¹) One could call these accordingly "imaginipetal" hormones, contrary to the metamorphosisrestraining corpus allatum hormone, for which WIGGLESWORTH recently suggested the designation "nymphal" (probably better "larval") hormone. [extra note added to translation: "imaginipetal" – organs that function only in the imago (wings, reproductive organs)]

I already had similar considerations previously (1942b, S. 179) regarding the early "pupal" development and the continuous "imaginal differentiation" following it. It seemed to me most probable that the driving hormones of the "early pupal" and the "imaginal" are the same.

We thereby empirically approach, presently in the context with *Drosophila*, the determination of the view derived by WIGGLESWORTH and FUKUDA already mentioned in the Introduction from observations of a bug and a butterfly, according to which to the same metamorphosis-promoting hormones are effective on all larval stages. It is still pointed out that we possess ourselves with the beginnings of the "internal" metamorphosis of the holometabolen *Drosophila* larva a process parallel to that occurring "outside" with the gradually approaching the adult changes at each molt of the heterometabolous bug.

SUMMARY

1. Ring glands of the first and early second larval stadia release typical pupating processes when they are implanted in isolated abdomens of the third larval stage. Here imaginal differentation can follow next.

2. Ring glands taken at different times deliver soon after transplantation in isolated abdomens different quantities of metamorphosis-promoting hormones. The ring glands of the same stages show also appropriate differences in effect with implantation into different age unligated larvae. In contrast, if the implantation takes place into equivalent age host larvae, then a provable influence on the metamorphosis is absent.

3. In isolated abdomens, developmental processes which normally take place in the second half of the second larval stadium and during the entire third larva stadium can be promoted in the eye antenna imaginal discs by implanted ring glands. The promotion takes place whether the host abdomens are in a larval or a pupal condition.

4. Also in unligated pupated hosts young eye antenna imaginal discs show first a typical larval advancement. To these a normal differentiation of the eye antenna imaginal discs is completed if the latter are transplanted again into unligated larvae.

5. The larval development of testicles can be likewise promoted by implanted ring glands. The same applies itself for the following early testicle

development. A hormone controlling spermiogenesis is is hereby uncovered for the first time, with *Drosophila*.

6. Transplantations of different ring gland sections show that probably the hormones of the [prothoracic gland portion of the ring gland called] thigh cells promote "larval" development of the eye antenna discs and testicles on the one hand, and puparium formation using "actual" metamorphosis process on the other hand. Furthermore, a remarkable acceleration of the eye antenna development results in the case of transplantation of thigh pieces [from which the corpora allatal cells have been excised away].

7. In the Discussion, first the possibility of a meaning of the metamorphosispromoting hormones for the larval molt is assessed. Furthermore a hypothesis is set up, according which to the "larval" development of the eye antennal imaginal discs and the testicle is the beginning of the "internal" metamorphosis, that is promoted by the same hormones of the thigh cells as do promote puparium formation during "actual" metamorphosis.

[English translation () by Davy Jones, Professor of Toxicology, University of Kentucky, Lexington, KY 40536, May 2006, made with the written permission of the author Margueritte Vogt, from her original German language text published in *Biologisches Zentralblatt* 1943, 63: 395-446,]