THE GROWTH OF THE GONADS IN *DROSOPHILA MELANOGASTER*

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INTRODUCTION

As shown by DOBZHANSKY and BRIDGES (1928), triploid intersexes in Drosophila may be considered as individuals developing up to a certain moment as males and thereafter as females. The earlier or later occurrence of the turning point in the development of an intersex determines the production of an individual manifesting relatively more male or relatively more female characteristics. If the turning point occurs early in the development, intersexes are produced having more female and less male characteristics. Conversely, if the turning point occurs late, the resulting intersexes become more male-like. In the extreme cases, that is, in cases when the turning point lies very early or very late in the development, intersexes are produced which are very similar to normal females or to normal males in appearance.

On the basis of these data BONNIER (1929) suggested the possibility of finding in the development of the normal females and males a phenomenon corresponding to the turning point of intersexes. The normal sexes in Drosophila differ from each other and from the intersexes by the altered balance of the female-determining and the male-determining factors. According to BRIDGES (1925), the female-determining factors are localized predominantly in the X-chromosomes, while the male-determining factors are localized mainly in the autosomes of Drosophila. Since both the X-chromosomes and the autosomes are present in the nuclei of normal females and males, both female-determining and male-determining factors are present in the germ-plasm of the normal sexual forms. Normal males may be, therefore, considered as individuals in which the turning point in

the development does not occur until the imaginal stage is reached, or until the end of their life. On the other hand, a turning point might be present in the earliest development of the normal females.

In order to secure data which might have some bearing on the problem raised by BONNIER, a study of the development and of the rate of growth of the gonads in females and males of Drosophila was undertaken. The size of the gonads was measured in larvae of different ages, beginning with those just hatched from the eggs, then through stages fully mature, and in young pupae. In older pupae the rapid differentiation of the tissues composing the gonads, and the alteration of the form of these organs make measurements difficult for the accurate description of the processes of growth taking place at that time.

MATERIAL AND METHODS

The growth of the gonads has been studied in wild-type flies belonging to the "Florida" stock of *Drosophila melanogaster* which was brought to Leningrad by Professor H. J. MULLER in 1922. Since the studies of growth require a thorough control of the age of the material under investigation, pains were taken to secure larvae and pupae of exactly known age that had developed under the same environmental conditions.

Fertilized females, from 4 to 8 days old, were placed in glass tubes containing a slide covered with a thin film of culture medium (raisin-agar sprinkled with a solution of commercial yeast). After 2 to 3 hours the slides with eggs deposited on the surface of the medium were removed from the tubes, and the eggs were carefully transferred one by one to the surface of a new slide covered by a still thinner film of raisin-agar, which was sufficiently homogenous and transparent to make the eggs easily noticeable. These slides were kept in a moist chamber in an incubator at 27.0 ± 0.3°C.

In order to obtain larvae of the same age, the slides were examined after 18 to 20 hours, and the larvae hatched from the eggs in the course of this time were discarded, since freshly laid eggs sometimes contain embryos in very different stages of development. Even fully formed larvae may be present in the freshly laid eggs (HUETTNER 1923). Thereafter the slides were examined regularly at one-hour intervals, and the freshly hatched larvae transferred to small crystallizing dishes provided with a standard amount of food (25cc of raisin-agar and 3 drops of 20 percent solution of yeast). Not less than 40 and not more than 50 larvae were placed in one crystallizing dish.

The further development of the larvae took place in the same incubator at 27°. Every 12 hours some of the larvae were extracted from the food, dissected, and the size of the gonads was measured. Their age was, therefore, known with an error not exceeding $\pm\frac{1}{2}$ hour.

The age of the pupae was controlled by a similar method. Cultures containing fully grown larvae were inspected at one-hour intervals, and freshly formed pupae were transferred individually to the surface of moist filter-paper. The development of pupae took place also at 27°C. Every 6 hours some of the pupae were dissected and the size of their gonads measured.

The dissection of the larvae and pupae was performed under a binocular microscope, in a drop of physiological salt solution, by means of sharp preparation-needles. The gonads sufficiently isolated from the fat-body were measured in units of an eyepiece-micrometer. Only one gonad was measured in each individual. Since the gonads of larvae and young pupae have the form of a nearly ideal ellipsoid, two measurements are sufficient for the characteristic of the size of a gonad. These measurements are the longer and the shorter diameters of the ellipsoid, and were made on each of the gonads studied. In the following tables the dimensions of the gonads are represented in terms of the units of the eyepiece-micrometer, one unit being equal to 1.12μ.

GENERAL CHARACTERISTICS OF THE LARVAL AND PUPAL GONADS

In the freshly hatched larvae of both sexes the gonads already represent well formed separate bodies which may be isolated from the surrounding tissues. The larval gonads lie in the body cavity surrounded by the fat-body, with which they are connected by very thin tracheal branches. The gonads are easily noticeable among the masses of fat-body because the tissue of the gonads is transparent and has a high refractive index, while the fat-body is not so transparent and is gray in color.

The sex of the gonads can be determined in freshly hatched larvae. The female gonads are considerably smaller in size than the male gonads, and are also different in their relation to the fat-body. The female gonad is covered by the fat-body tissue, while the male gonad lies more free, being only bordered by the fat-body. The indifferent stage of the development of the gonads can not, therefore, be found in larvae. It remains to be determined whether there is a still earlier stage at which the sex of a gonad can not be distinguished either by an anatomical or by a histological investigation.

The above description of the gonads of the freshly hatched larvae fits almost equally well for the gonads of the larvae of all the older stages. The

form of the gonads becomes more oblong-ellipsoidal with age. This is especially true in respect to the male gonad. Besides this and the rapid increase in size the gonads undergo little or no change during the whole larval stage. In the 6-hour larvae the male gonads are already so large that they begin to be seen through the body-wall of the living larva. This fact gives the possibility to determine the sex of the larvae without dissection. The accuracy of such determination has been checked by classifying the sex of 6 to 10 hour larvae and raising the females and males in separate bottles. The sex of the mature flies which appeared in these bottles was in accord with the expectation.

The genital ducts (oviducts, *vasa deferentia*) are represented in larvae by an imaginal disc lying ventral to the end of the intestine. No connection of any kind between the imaginal discs and the gonads is observable in larval stages. After the pupation the imaginal discs begin to grow very rapidly, being gradually transformed into the genital ducts. The structure of the gonads also undergoes profound changes in the pupal stage. The rudiments of the egg-strings are noticeable in the female gonad of very young pupae. In 6 to 12 hour pupae these rudiments are plainly visible. True egg-strings are, however, formed much later, in the second half of the pupal period. At 36 to 42 hours after pupation the female gonads become attached to the oviducts. In the male gonad there is observed a rapid differentiation of the tissue composing the gonad. In the 24-hour pupae bundles of developing spermatozoa may sometimes be observed in the male gonads. In the 30-hours old pupae the male gonads begin to lose their regular ellipsoidal shape; their longer diameter begins to grow much faster than the shorter diameter, the gonads become oblong, and gradually assume the spiral form characteristic of the adult testis. At the same age the male gonads become attached to the *vasa efferentia*.

Since after pupation the histological differentiation of the tissues composing the gonads becomes very rapid, the rate of growth of the female and male gonads is strictly comparable only in the larvae and very young pupae. I studied the dimensions of the gonads up to the 30-hour pupae. In still older stages the measurements of the two diameters of the gonads become insufficient for even an approximate description of the process of growth taking place at that time.

THE GROWTH OF THE LINEAR DIMENSIONS OF THE GONADS

As mentioned above, the size of the longer and the shorter diameters of the female and the male gonads was measured at 12-hour intervals in the larval stage and at 6-hour intervals in the pupal stage (pupation occurs at

TABLE 1

Growth of the linear dimensions of the female gonads.

	AGE IN HOURS	LONGER DIAMETER				SHORTER DIAMETER				n
		$M \pm m$	σ	Lim	Diff.	$M \pm m$	σ	Lim	Diff	
Larvae	0	2.88±0.10	0.51	2 –4		2.52±0.08	0.39	1½–3		25
					0.16±0.14				0.16±0.10	
Larvae	12	3.04±0.09	0.44	2 –4		2.68±0.09	0.46	2 –3		25
					0.96±0.17				0.72±0.14	
Larvae	24	4.00±0.14	0.69	3 –5		3.40±0.13	0.63	2 –5		25
					0.20±0.20				0.40±0.17	
Larvae	36	4.20±0.13	0.57	3 –5		3.80±0.10	0.49	3 –5		25
					0.44±0.17				0.56±0.14	
Larvae	48	4.64±0.12	0.62	4 –6		4.36±0.10	0.48	4 –5		25
					0.96±0.17				0.72±0.14	
Larvae	60	5.60±0.14	0.69	5 –7		5.08±0.08	0.39	4 –6		25
					1.92±0.20				1.24±0.14	
Larvae	72	7.52±0.14	0.70	6 –9		6.32±0.12	0.61	5 –8		25
					1.92±0.24				1.60±0.22	
Larvae	84	9.44±0.20	0.98	8–12		7.92±0.19	0.97	6–10		25
Pupae	0	13.12±0.14	0.71	12–14		9.88±0.14	0.71	9–12		25
					3.84±0.24				1.84±0.22	
Pupae	12	16.96±0.21	1.04	15–19		11.72±0.17	0.87	10–14		25
					0.02±0.28				1.08±0.24	
Pupae	18	16.98±0.19	1.23	15–19		12.80±0.18	1.18	11–15		44
					0.18±0.32				1.88±0.28	
Pupae	24	17.16±0.25	1.28	15–20		14.68±0.23	1.16	12–18		25
					0.58±0.35				1.26±0.32	
Pupae	30	17.74±0.25	1.46	15–21		15.94±0.22	1.30	14–20		34

TABLE 2

Growth of the linear dimensions of the male gonads.

	AGE IN HOURS	LONGER DIAMETER				SHORTER DIAMETER				n
		$M \pm m$	σ	Lim	Diff	$M \pm m$	σ	Lim	Diff	
Larvae	0	4.30±0.06	0.35	4 –5		3.89±0.05	0.27	3 –4		30
					1.58±0.14				1.27±0.10	
Larvae	12	5.88±0.13	0.65	5 –7		5.16±0.11	0.54	4 –6		25
					2.00±0.20				1.00±0.17	
Larvae	24	7.88±0.17	0.86	6–10		6.16±0.12	0.61	5 –7		25
					3.16±0.22				2.24±0.17	
Larvae	36	11.04±0.16	0.82	10–13		8.40±0.14	0.69	7–10		25
					2.92±0.24				2.84±0.20	
Larvae	48	13.96±0.19	0.94	12–16		11.24±0.13	0.65	10–12		25
					3.48±0.32				2.56±0.28	
Larvae	60	17.44±0.26	1.30	15–19		13.80±0.25	1.23	11–16		25
					3.84±0.39				2.52±0.41	
Larvae	72	21.28±0.29	1.46	19–25		16.32±0.33	1.64	13–20		25
					4.40±0.23				4.16±0.45	
Larvae	84	25.68±0.38	1.89	22–29		20.48±0.30	1.50	18–23		25
Pupae	0	27.80±0.34	1.72	25–30		21.24±0.31	1.53	18–23		25
					1.83±0.43				2.68±0.42	
Pupae	12	29.63±0.28	1.64	27–33		23.92±0.29	1.71	20–27		35
					2.13±0.47				1.81±0.41	
Pupae	18	31.76±0.38	2.04	28–37		22.11±0.29	1.56	20–26		29
					1.76±0.54				1.17±0.46	
Pupae	24	33.52±0.38	1.92	30–37		23.28±0.36	1.81	20–28		25
					4.28±0.58				0.48±0.56	
Pupae	30	37.80±0.44	2.22	35–43		23.76±0.42	2.12	20–30		25

the temperature used at $88.6 \pm .07$ hours after hatching from the eggs, the extrusion of the anterior spiracles being taken as the moment of pupation).

The results of the measurements are presented in tables 1 and 2. Besides the mean values and their mean errors ($M \pm m$) there are included in these tables also the observed limits of variation (Lim), the standard deviations (σ) and the number of individuals studied (n). The columns marked "Diff" present the differences observed between the size of the longer (or the shorter) diameter of the gonads observed in successive ages, that is, the amount of increase of the size of the respective diameter during the given interval of time. As is shown by the figures included in tables 1 and 2, these differences are in most cases larger than their trebled errors, that is, they are statistically significant. This rule, however, does not hold for

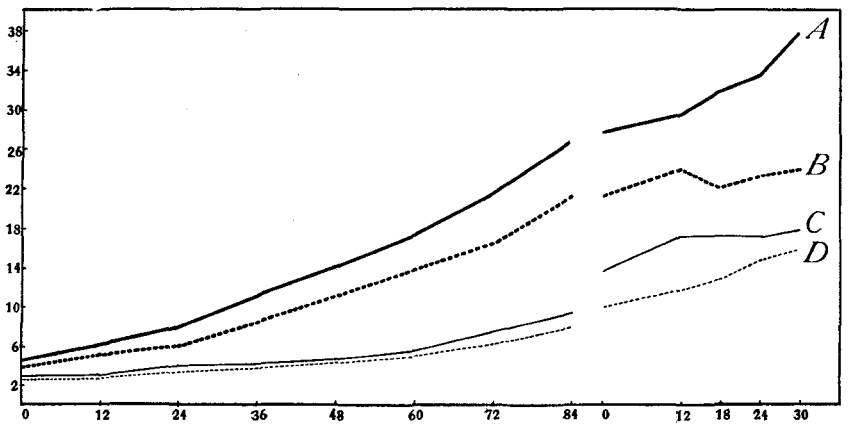

FIGURE 1.—Growth of the two diameters of the gonads. A, longer diameter of the male gonad; B, shorter diameter of the male gonad; C, longer diameter of the female gonad; D, shorter diameter of the female gonad. Horizontal axis—age in hours; vertical axis—size in units of the micrometer.

the younger ages of the female larvae, where the 12-hour intervals give in most cases statistically uncertain increase of the size of the gonads. This fact may be due either to the slow rate of growth of the female gonads in the beginning of the larval stage, or to the inaccurate determination of the size of the gonads. Indeed, the young female gonads are so small that it is difficult to measure them as accurately as the male gonads or the older female gonads.

Data on the growth of the female and male gonads may be represented also in the form of the curves shown in figure 1. The shape of these curves suggests that the rate of growth of the female gonads is somewhat different from the rate of growth of the male gonads. The male gonads of the freshly

hatched larvae are markedly larger than the female gonads of the same age. Furthermore, the size of the male gonads increases more rapidly than that of the female gonads. The curves of growth of the female gonads run nearly parallel to the horizontal axis of the diagram for the first half of the duration of the larval stage. In older larvae the increase of the size of the female gonads proceeds more rapidly. After pupation the shorter diameter of the male gonads grows less rapidly than the longer diameter. This fact is demonstrated by the rapid divergence of the curves representing the growth of the two diameters of the male gonads after pupation. The begin-

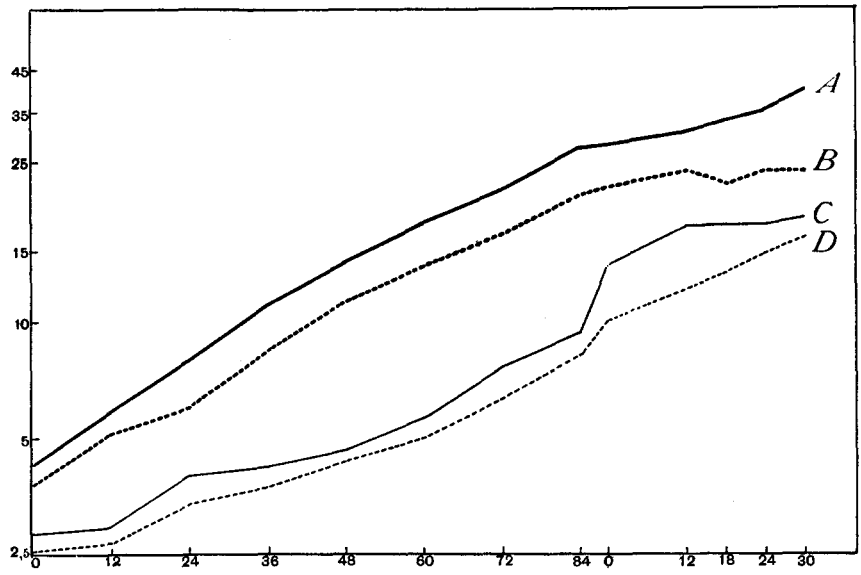

FIGURE 2.—Logarithmic curves representing the growth of the two diameters of the gonads. A, longer diameter of the male gonad; B, shorter diameter of the male gonad; C, longer diameter of the female gonad; D, shorter diameter of the female gonad. Horizontal axis, age in hours.

ning of this divergence corresponds apparently with the beginning of the rapid differentiation of the tissues composing the male gonads (see above). No such sharp divergence is observed for the rate of growth of the longer and the shorter diameters of the female gonads.

The differences in the rate of growth of the female and the male gonads become more evident if the growth is represented by logarithmic curves (figure 2); in this figure the vertical axis represents the logarithms of the diameters of the gonads. The curves of growth of the longer and the shorter diameters of the male gonads are similar in shape. The angle formed by these curves with the horizontal axis of the diagram progressively diminishes with age and finally tends to approach zero (at least for the

shorter diameter). This fact shows the progressive decrease of the rate of growth of male gonads with age.

It is well known that the rate of growth of a whole organism or of a separate organ tends to decrease with age. The curves of growth of the female gonads are different. The angle formed by these curves and the horizontal axis of the diagram increases with age at least during the whole larval stage. That is to say, the rate of growth of the female gonads shows a progressive increase with age instead of a decrease. The increase of the rate of growth with age is observed in general less frequently than the opposite condition. This fact suggests that the growth of the female gonads takes place under some influences which distort the usual shape of the growth curve.

THE GROWTH OF THE VOLUME OF THE GONADS

The male as well as the female gonads have a regular ellipsoid shape during the whole larval and the beginning of the pupal stage. The volume of an ellipsoid can be easily calculated according to the formula $V = 4/3\pi a\, b^2$, where a is the longer and b is the shorter half-axis of the ellipsoid. The volumes of the female and the male gonads calculated according to this

TABLE 3
Growth of the volume of the gonads.

AGE IN HOURS	LARVAE							
	0	12	24	36	48	60	72	84
Females	9.57	11.43	24.20	31.74	46.16	75.6	157.2	309.9
Males	33.88	78.79	156.49	407.67	923.10	1738.1	2966.1	5636.9
	PUPAE							
	0	12	18	24	30			
Females	670.2	1219.2	1455.9	1935.3	2358.9			
Males	6563.3	8869.4	8792.6	9507.0	11117.0			

formula are presented in table 3. Figure 3 shows the logarithmic curves representing the growth of the volume of the gonads.

The consideration of the curves shown in figure 3 leads to the same conclusions as the consideration of those shown in figure 2. The angle formed by the curve of growth of the male gonads with the horizontal axis of the diagram decreases rather regularly with age. The angle between the curve of growth of the female gonads and the horizontal axis of the diagram does not show the tendency to decrease with age, but increases pro-

gressively, at least until the pupal stage is reached. Taken as a whole, the curve of growth of the male gonads is convex toward the upper side of the diagram, while the curve of growth of the female gonads is somewhat convex toward the lower side of the diagram. In other words, the rate of growth of the male gonads decreases with age, while the rate of growth of the female gonads increases with age, at least until the pupal stage is reached. Only after pupation does the rate of growth of the female gonads cease to increase, and subsequently it begins even to decrease with age.

It may be suggested that the difference in the shape of the growth curves observed between the female and the male gonads is due to the fact that

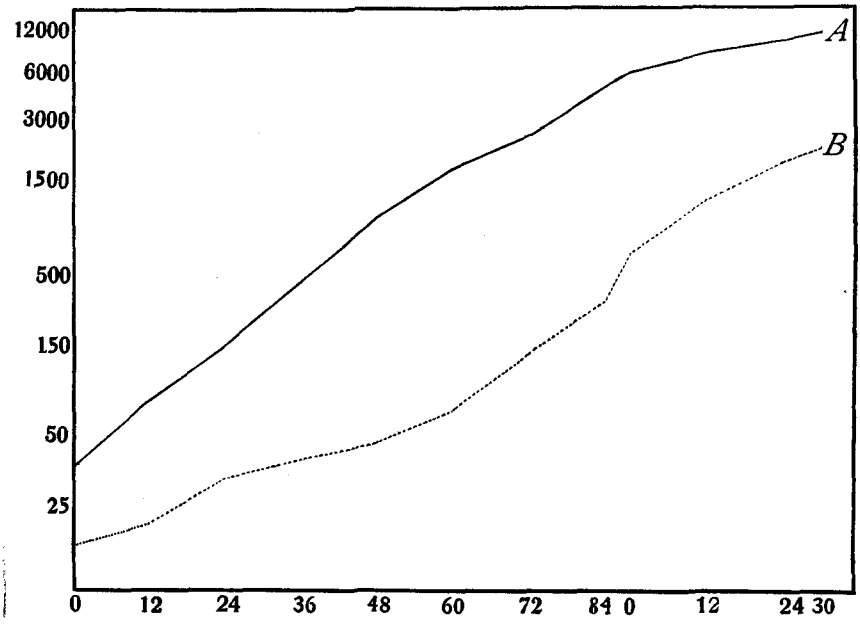

FIGURE 3.—Logarithmic curves representing the growth of the volumes of the gonads. A, male gonad; B, female gonad. Horizontal axis—age in hours.

the absolute size of the male gonads is larger than that of the female gonads. Such an explanation is, however, unsatisfactory. If the volume of the female and male gonads observed in 30-hour old pupae is taken as 100, and the volumes observed in other stages are expressed in percentages of this value, the curves of growth assume the shape shown in figure 4. These curves show clearly that the rate of growth of the female gonads is suppressed in the beginning of the larval period and becomes more intensive toward its end.

In order to ascertain once more the existence of the inferred difference between the rate of growth of the female and the male gonads the formula

of the rate of growth proposed by SCHMALHAUSEN (1928) may be used. According to this formula the rate of growth (C_v) of the volume of an organ during the time interval $t_1 - t$ is equal to:

$$C_v = \frac{\lg v_1 - \lg v}{0.4343\ (t_1 - t)},$$

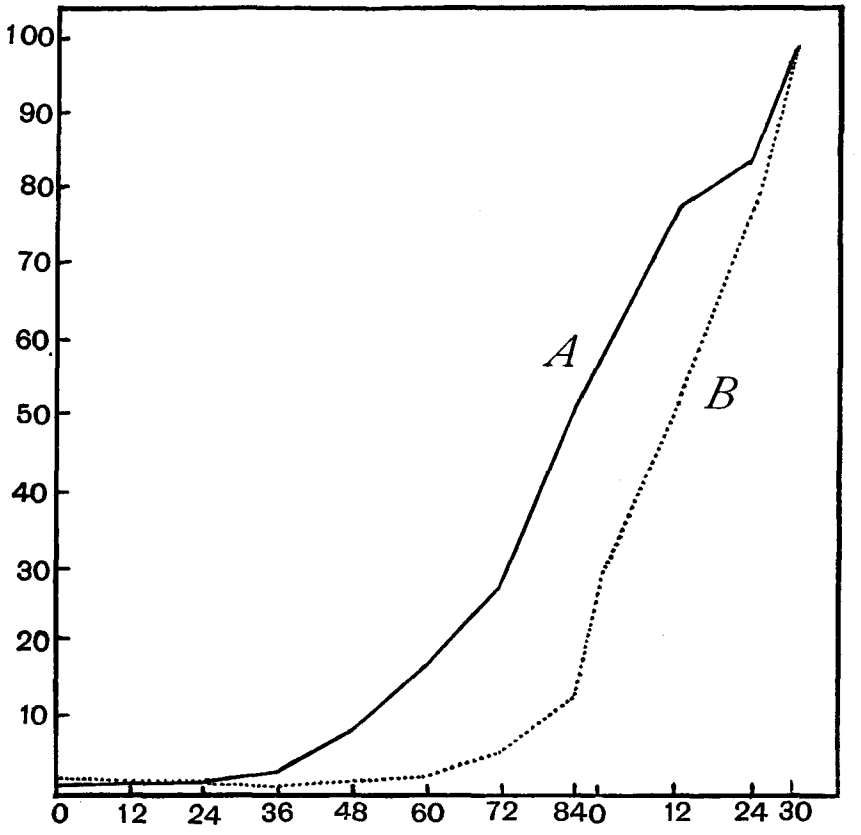

FIGURE 4.—Volume of the gonads in different ages (horizontal axis), represented in percentages of the volume attained at 30 hours of the pupal life (vertical axis). A, male gonad; B, female gonad.

TABLE 4

Coefficients (C_v) of the rate of growth of the volumes of the gonads.

AGE IN HOURS	LARVAE							PUPAE	
	0–12	12–24	24–36	36–48	48–60	60–72	72–84	0–12	12–24
Females	22.9	40.6	25.2	27.9	31.4	40.0	37.8	34.9	30.4
Males	44.6	38.1	50.0	43.4	36.1	32.7	36.5	25.9	20.6

where v and v_1 represent the volumes of the organ at the moments t and t_1 respectively. The values of C_v for the growth of the female and male gonads are shown in table 4 and figure 5. During the first 24 hours of the larval life the value of C_v fluctuates irregularly. The significance of these fluctuations is, however, doubtful, since the accuracy of determination of the size of the gonads is at its lowest just in these early stages of larval development. But beginning with the 24-hour old larvae the rate of growth of the male gonads steadily decreases, while the rate of growth of the female gonads increases with age. At the beginning of the development the rate of growth of the male gonads is higher than that of the female gonads; at the end of the larval period and in the pupal period the rate of growth

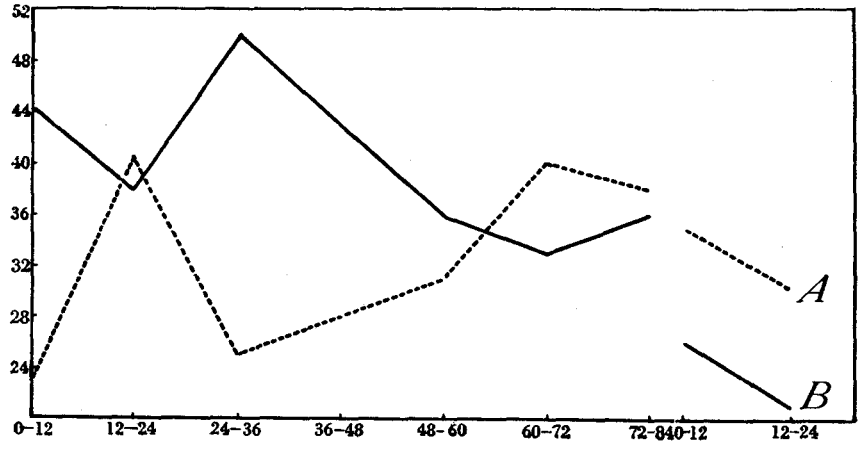

FIGURE 5.—Coefficients of the rate of growth (C_v). A, male gonad; B, female gonad. Horizontal axis—time intervals. Vertical axis—coefficients C_v.

of the female gonads exceeds that of the male gonads. After pupation the rate of growth of the female gonads begins to decrease with age, being, however, higher than the rate of growth of the male gonads.

SUMMARY

1. The sex of the gonads of freshly hatched larvae can be distinguished with complete certainty. The male gonads are larger in size than the female gonads, and show a different relation to the surrounding fat-body tissues.

2. The rate of growth of the diameters and of the volumes of the gonads has been studied. In males the rate of growth gradually decreases with age. In females the rate of growth of the gonads increases with age during the larval life. After the pupation there is observed a decrease of the rate of growth also in females.

3. During the first half of the larval stage the rate of growth of the female gonads is lower than the rate of growth of the male gonads. In the second half of the duration of the larval stage and in pupae the conditions are reversed.

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