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LARVAL STOP, DELAYED DEVELOPMENT AND SURVIVAL IN OVERCROWDED CULTURES OF *DROSOPHILA MELANOGASTER*: EFFECT OF UREA AND URIC ACID

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Abstract—Uric acid and urea added to non-crowded cultures of *D. Melanogaster* are able to reproduce the larval stop (cessation in development) detected in highly competitive situations. The quantitative analysis of media as well as of larvae and pupae reveals the presence of both compounds as natural waste products of nitrogen metabolism in *Drosophila*. The nature of their effect is discussed in terms of larval intoxication as a mechanism which may account for the effects usually observed in crowded cultures: development delay, lower survival and also larval stop (which can only be detected by interrupting the competitive process by an overfeeding technique).

Key Word Index: *Drosophila melanogaster*, larval competition, development time, survival, larval stop, intoxication

INTRODUCTION

In a series of experiments carried out with different strains of *D. melanogaster*, using an overfeeding technique (Ménsua and Moya, 1983), it has been demonstrated that extreme competition gives rise to a developmental stop in third-instar larvae. Two kinds of explanations are both possible of this stop in development: (i) the waste products of larvae at high concentrations could inhibit larval development by disturbing normal metabolism. Though no metabolic residue has been directly reported as a cause of phenomena affecting the survival and developmental time, the effects of larval biotic residues on viability as inter- and intraspecies and genotypes interactions have been reported in the literature (Budnik and Brncic, 1974, 1976; Dawood and Strickberger, 1969a,b; Huang *et al.*, 1971; Palabost, 1973; Weisbrot, 1966). In all of them the effect of media conditioned by larvae of different strains and species were examined, but in none of them was the influence of specific excretory products on viability and mean developmental time studied (ii) An alternative hypothesis which has been generally accepted as an explanation of the results in high-competition cultures, supposes that overcrowding causes a scarcity of certain compounds necessary to further development. In this respect, it is worth mentioning that the following products which have been studied: the sterol deficiencies which produce extensive mortality either at the larval-last instar or at the pupal stage (Bos, 1979; Bos *et al.*, 1977; Heed and Kircher, 1965); the absolute requirement of biotine in *D. melanogaster* as demonstrated by Erk and Sang (1966). Also the effects of choline scarcity on the growth and development of *D. melanogaster* were reported by Geer and Vovis (1965). All of these products are growth limiting in critical phases and share the common feature of being required in conspicuous amounts.

In the present work a series of experiments has been carried out which leads to the conclusion that uric acid, urea and possibly other waste products (not excluding some scarcity of certain metabolites) account not only for the results usually observed in survival and development time in competition conditions, but also for the developmental stop in 3rd instar larvae.

MATERIAL AND METHODS

An isogenic Oregon-R strain was used for all experiments. This strain has been maintained at $25 \pm 1°C$ by brother × sister crosses since its arrival at our Department in March 1982. All the experiments were carried out at $18 \pm 0.5°C$ and 85% r.h.

Three kinds of vials were employed: small tubes (4 × 0.8 cm) supplied with 0.5 ml of Lewis' medium, large tubes (10 × 2.7 cm) supplied either with 5 ml of Lewis' medium or with 10 ml of the same medium but in an inclined position for overfeeding, and bottles with inclined food (25 ml) large enough to permit the large tubes to be introduced into them.

Flies aged from 5 to 6 days were placed in vials covered with watch glasses which contained agar, acetic acid, ethyl alcohol and live yeast for the eggs laid. The glasses were incubated at $25 \pm 1°C$ for 24 h and then the hatched larvae, aged 2 ± 2 h, were selected. The methodology employed for each type of experiment was as follows:

I. Control of overfeeding in crowded media at 18°C

According to the technique described by Ménsua and Moya (1983) and Moya and Ménsua (1983b) developmental stop, survival and mean devel-

opmental time in days was measured. Because of the lengthening of development at 18°C, the overfeedings were carried out at 13th, 17th, 21st, 25th and 29th day.

II. Conditioned media

Vials containing 5 ml of food were seeded with 70 larvae. At different times (4th, 6th, 8th, 10th, 12th, 14th and 16th day from the starting day) larvae were extracted, washed in distilled water and seeded again in small tubes with 0.5 ml of food which had previously harboured 70 larvae for 13 days. The media so conditioned were free of larvae which had been extracted by the overfeeding technique on the 13th day of culture. The development time and survival of the emerged adults were determined. A total of 10 replicates were made. At the same time the larval instar of larvae which were seeded in the conditioned media was determined by jaw morphology, as well as their ability to pupate in the absence of food.

III. Experiments with urea and uric acid

In these experiments two kinds of methods were employed:

A. Effect of urea or uric acid. In order to determine the viability and mean development times at different concentations of urea or uric acid, 70 larvae were seeded in 5 ml of Lewis' medium. A total of 5 replicates were made. The concentrations employed were 2, 4, 6, 8, 10, 12 and 14 mg/ml in the case of urea and 4, 8, 10 and 12 mg/ml for uric acid. Owing to the poor solubility of uric acid in Lewis' medium, a homogenate was made in order to distribute it into the media.

B. Overfeeding experiments of media with urea or uric acid. Seventy larvae were seeded in each of 6 tubes with 5 ml of Lewis' medium containing 10 mg/ml either of urea or uric acid. The tubes were distributed as follows: 5 tubes which were overfed at different times (13th, 17th, 21st, 25th and 29th day) by placing them in bottles with inclined food of Lewis' medium, and a control tube was not overfed. Due to the non-competition situation the larvae did not migrate spontaneously to the bottles and were introduced carefully with the aid of a brush. A total of ten replicates were made.

IV. Quantitative analysis of urea and uric acid

A. Analysis in culture media. Seventy larvae were seeded in 0.5 ml of Lewis' medium in small tubes. At different times (5th, 9th, 13th, 17th, 21st, 25th and 29th day from the start of the experiments) the tubes were overfed. In this way media free of larvae were obtained and subjected to quantitative analysis of urea and uric acid. The procedure was the following: the media were homogenized with 1 ml of sodium acetate 0.1 M, centrifuged twice; first into 10 ml tubes at 4000 rpm for 5 min in order to eliminate the greater part of the agar residues, and secondly in small Eppendorf tubes at 4000 rpm for 10 min. The supernatant were analysed for urea and uric acid. In the case of urea and acetyl-monoxime assay was employed (Lemar and Bootzin, 1957). For uric acid the Fe^{3+} reduction was applied (Collins *et al.*, 1959). A total of 5 replicates was made.

B. Analysis of larvae and pupae. At different times (9th, 13th, 17th, 21st, 25th and 29th day) throughout the cultures, larvae and pupae were extracted, washed in distilled water, dried on filter paper and homogenized in sodium acetate (0.1 M) in order to solubilize the uric acid. Larvae and pupae were chosen in groups of 20 larvae or pupae using a total of 90 μl of sodium acetate at a final concentation of $\frac{1}{10}$ approx. The urea and uric acid contents were determined using the same procedure as mentioned above. A total of 5 replicates were made.

RESULTS

I. Control of overfeeding in crowded media at 18°C

Table 1 represents the survival and mean development times of inner and outer populations. As can be seen the results are very similar to those found by Ménsua and Moya (1983) at 25°C. Nevertheless they show a lower survival and a longer mean development time. The larval stop is also detected ($b = 1.03$ vs $b = 1.04$ in the Oregon-R at 25°C). The number of days to attain the adult stage from the day of overfeeding is higher ($a = 12.22$ as shown in Table 1 vs $a = 8.51$ in Oregon-R at 25°C).

II. Conditioned media

Table 2 shows age of larvae seeded, larval stage when larvae are seeded, percentage of pupation in the absence of food, average number of seeded larvae, viability and mean development times of larvae which were seeded into the conditioned media. The ability to attain pupation in the absence of food was made following Church and Robertson (1966). As can be seen from this table, larvae bred for 4 or 6 days in non-crowded conditions (70 larvae in 5 ml of Lewis' medium) were unable to attain the adult stage and, in the case of 4-day old larvae, even to pupate. The larvae died in the third instar as revealed by jaw

Table 1. Experiment I—Mean survival (S) and mean developmental time (MDT) with standard errors in inner, outer and total populations throughout overfeeding of crowded media

	Inner		Outer		Total
Overfeeding	S	MDT	S	MDT*	S
Control 5 ml	—	—	55.0 ± 4.0	22.08 ± 0.42	55.0 ± 4.0
13	2.0 ± 0.8	26.65 ± 0.91	54.0 ± 1.8	26.18 ± 0.26	56.0 ± 1.6
17	3.0 ± 0.6	27.00 ± 0.92	46.0 ± 2.7	29.73 ± 0.22	49.0 ± 2.2
21	8.1 ± 0.9	26.72 ± 0.45	38.5 ± 3.6	33.20 ± 0.34	46.6 ± 3.5
25	12.1 ± 1.4	27.46 ± 0.71	16.8 ± 2.4	37.51 ± 0.48	28.9 ± 2.5
29	11.1 ± 1.2	27.81 ± 0.45	5.6 ± 1.4	42.95 ± 0.70	16.7 ± 1.9
Control 0.5 ml	12.1 ± 1.4	28.13 ± 0.48	—	—	12.1 ± 1.4

*Regression of MDT over the overfeeding (in outer populations): $a = 12.22$, $b = 1.03$ and $R^2 = 0.996$.

Table 2. Experiment II—Conditioned media: percentage of viability (V) and mean developmental time (MDT)

Days when the larvae were extracted	Larval stage when the larvae are transferred into conditioned media (%)	Percentage of pupation in the absence of food	Average number of transferred larvae (ten replicae)	V	MDT
4	100.0 (2nd)	0	30.0	2.08 A* 13.30 P** 84.62 L***	40.38 ± 1.63
6	92.8 (2nd) 2.4 (2nd–3rd) 4.8 (3rd)	0	52.0	1.94 A 30.19 P 67.87 L	30.00 ± 0.00
8	50.0 (2nd) 12.0 (2nd–3rd) 38.0 (3rd)	0	53.0	41.52 A 2.04 P 56.44 L	27.21 ± 0.52
10	100.0 (3rd)	100	56.0	74.36 A 1.27 P 24.37 L	26.71 ± 0.16
12	100.0 (3rd)	100	65.0	75.50 A 1.04 P 23.46 L	25.44 ± 0.16
14	100.0 (3rd)	100	56.0	75.00 A 0.04 P 24.06 L	24.66 ± 0.23
16	100.0 (3rd)	100	45.0	80.80 A 0.07 P 19.13 L	25.50 ± 0.12
Control	—	—	—	83.14 A 0.00 P 16.86 L	25.11 ± 0.18

*A = Adults; **P = dead pupae; ***L = 3rd dead larvae.

analysis. Eight-day old larvae were delayed in their development, compared to the controls, and a large number failed to emerge as adults: the jaw analysis showed that approximately half of the larvae were third instar and the other were second instar or at the moult stage. None of these larvae was able to pupate when starved. Ten-day old larvae suffered a slight development delay and some pupae failed to emerge as adults. These larvae were all third instar and were able to pupate in the absence of food. From the 12th day on, larvae did not suffer significant delays and pupal mortality decreased. Altogether, these results seem to support the existence of some excretory product(s) into the conditioned media, which impedes or delays the development into the adult state until a certain stage (12 days), after which larvae are not sensitive.

Media used for 13 days were chosen because of their high content in uric acid (see below part IV) while, nevertheless, appreciable amount of food remained.

III. Experiments concerned with urea and uric acid

A. Effects of urea and uric acid on development and survival.

The search for some waste products to account for the above results, led to the assay of urea (main waste product in the nitrogen metabolism of mammals) and uric acid (main waste product in insects) in non-competitive media (70 larvae in 5 ml of Lewis' medium). Tables 3 and 4 show the survival and developmental times at different concentrations of urea and uric acid respectively. As can be seen, the developmental times follow a linear regression. Survival does not follow this kind of regression as studied by Moya and Ménsua (1983a) describing a Gompertz's deterministic model of mortality. High concentrations of these products produce a delay similar to that found in the competition experiments (see Table 1).

The survival decreases with urea and uric acid concentrations, the reduction produced by urea being more drastic. The survival obtained with uric acid is closer to the overcrowded situations.

B. The overfeeding experiments in media supplemented with urea or uric acid. Once urea as well as uric acid has been shown to delay development, the following step was the attempt to reproduce the results obtained in crowded conditions by overfeeding non-competition media (70 larvae in 5 ml) supplemented with 10 mg/ml either urea or uric acid.

Table 3. Experiment IIIA—Mean survival (S) and mean developmental time (MDT) with standard errors in media supplemented with urea

Dose (mg/ml)	S	MDT*
0 (control)	56.2 ± 1.8	23.88 ± 0.37
2	41.8 ± 3.5	28.54 ± 0.66
4	39.4 ± 5.2	29.60 ± 0.54
6	37.2 ± 6.0	30.88 ± 0.30
8	26.4 ± 6.6	32.36 ± 0.16
10	9.6 ± 2.4	32.24 ± 0.24
12	1.0 ± 0.3	34.75 ± 0.85

*Regression of MDT over doses of urea: a = 25.74, b = 0.76 and $R^2 = 0.900$.

Table 4. Experiment IIIA—Mean survival (S) and mean developmental time (MDT) with standard errors in media supplemented with uric acid

Dose (mg/ml)	S	MDT*
0 (control)	66.1 ± 0.7	26.64 ± 0.12
4	50.4 ± 2.8	29.05 ± 0.15
8	45.5 ± 1.9	31.68 ± 0.13
10	39.2 ± 3.3	32.85 ± 0.19
15	33.0 ± 1.6	33.50 ± 0.28

*Regression of MDT over doses of uric acid: a = 27.18, b = 0.48 and $R^2 = 0.93$.

Table 5. Experiment IIIB—Mean survival (S) and mean developmental time (MDT) with standard errors in inner, outer and total populations throughout overfeeding of non-crowded media supplemented with 10 mg/ml of urea

	Inner		Outer		Total
Overfeeding	S	MDT	S	MDT*	S
Control 5 ml	—	—	55.8 ± 2.3	27.19 ± 0.31	55.8 ± 2.3
13	4.2 ± 0.9	33.49 ± 0.42	14.4 ± 0.8	35.20 ± 0.31	18.6 ± 1.2
17	1.7 ± 0.5	34.35 ± 0.69	16.1 ± 1.7	35.89 ± 0.48	17.8 ± 2.4
21	2.9 ± 1.0	33.76 ± 0.57	13.1 ± 1.1	36.90 ± 0.38	16.0 ± 1.7
25	8.3 ± 3.5	33.61 ± 0.61	3.9 ± 1.8	42.04 ± 1.56	12.2 ± 4.7
29	8.3 ± 3.1	33.90 ± 0.23	0.3 ± 0.2	50.00 ± 0.71	8.6 ± 3.2
Control 5.0 ml with urea	10.3 ± 1.2	33.87 ± 0.24	—	—	10.3 ± 1.2

*Regression of MDT over overfeeding (in outer populations): a = 21.24, b = 0.81 and $R^2 = 0.91$.

Table 6. Experiment IIIB—Mean survival (S) and mean developmental time (MDT) with standard errors in inner, outer and total populations throughout overfeeding of non-crowded media supplemented with 10 mg/ml of uric acid

	Inner		Outer		Total
Overfeeding	S	MDT	S	MDT*	S
Control 5 ml	—	—	58.6 ± 3.1	25.08 ± 0.71	58.6 ± 3.1
13	10.4 ± 1.3	28.51 ± 0.39	31.1 ± 2.2	28.29 ± 0.23	41.5 ± 2.1
17	12.7 ± 2.8	27.90 ± 0.83	26.2 ± 2.5	29.80 ± 0.30	38.9 ± 3.3
21	22.4 ± 3.6	28.61 ± 0.58	12.6 ± 2.4	32.33 ± 0.15	35.0 ± 3.8
25	38.9 ± 3.7	29.03 ± 0.53	1.3 ± 0.6	35.89 ± 0.22	40.2 ± 3.5
29	29.9 ± 4.1	29.90 ± 0.83	0.1 ± 0.1	38.00	30.0 ± 4.0
Control 5 ml with uric acid	34.4 ± 3.4	29.91 ± 0.32	—	—	34.4 ± 3.4

*Regression of MDT over overfeeding (in outer populations): a = 19.47, b = 0.64 and $R^2 = 0.99$.

Tables 5 and 6 show the survival and mean developmental times throughout the different overfeedings, in the media with urea and uric acid respectively. As can be seen, the results only roughly approach those found in crowded conditions (see Table 1). With urea media, in general higher mortality and a longer period of developmental time, especially in outer populations, can be seen. There is a linear regression between development times and overfeeding in media with urea as well as with uric acid, which indicates a development stop. With urea the stop becomes longer in the last overfeeding when the outer developmental times increase greatly (see Table 5). On the other hand, the uric acid produces a more limited development stop than that found with urea and even than that obtained in crowded conditions (see Table 6). The shorter stop detected with uric acid in non-competitive conditions is similar to previous results obtained with low densities (35 larvae in 0.5 ml of food, data not shown).

Altogether the results reveal the possibility of reproducing the larval stop in non-crowded conditons and by means of natural waste products.

IV. Analysis of urea and uric acid

A. Analysis in culture media. Table 7 shows the concentration of urea and uric acid at different times during culture. Nine points have been selected, the last five being the time of overfeeding: 5th, 9th, 13th, 17th, 21st, 25th and 29th day.

The dynamics of the uric acid concentration is complex with two maxima at 9–13th and 29th day, and minimum at 21st day (Fig. 1). These results clearly indicate the complexity of the process between excretion and ingestion of waste products. In the nine first days the concentration of uric acid rose quickly, reaching a peak at 9–13th day which can be explained in terms of an accumulation of waste products with the larval development. Nevertheless the larvae, from a given moment on, will begin to ingest their own waste products. From the 13th to the 21st day of culture the concentration of uric acid decrease, which can be explained as in increasing ingestion of uric acid by larvae, and the amount of excretion also should decrease. After the 21st day of culture there is a new increase in uric acid content, correlated with a large amount of pupation on the walls of the small tubes.

As regards urea, to the best of our knowledge this is the first report where urea is mentioned as an excretory product in *D. melanogaster*, though urea has been reported as a minor waste product in some uricotelic invertebrates (Thruszkowski and Chajkinown, 1935; Needham, 1935). The level of urea found in the culture media is low, as can be expected from an uricotelic insect whose main excretory product is uric acid. However, a striking increase is observed at exactly the time when uric acid concentration also increases. It is possible that the excretion of uric acid is being facilitated by its conversion into urea in the larvae.

Table 7. Experiment IVA—analysis of urea and uric acid in crowded media throughout the overfeedings

Overfeeding (days)	Urea (mg/100 ml)	Uric acid (mg/100 ml)
5	1.84 ± 0.59	9.44 ± 0.80
9	2.44 ± 0.95	25.57 ± 1.33
13	0.32 ± 0.20	26.48 ± 1.19
17	0.38 ± 0.17	19.85 ± 0.74
21	3.50 ± 1.55	7.89 ± 2.40
25	12.46 ± 2.09	13.85 ± 2.43
29	5.66 ± 1.80	19.70 ± 1.92

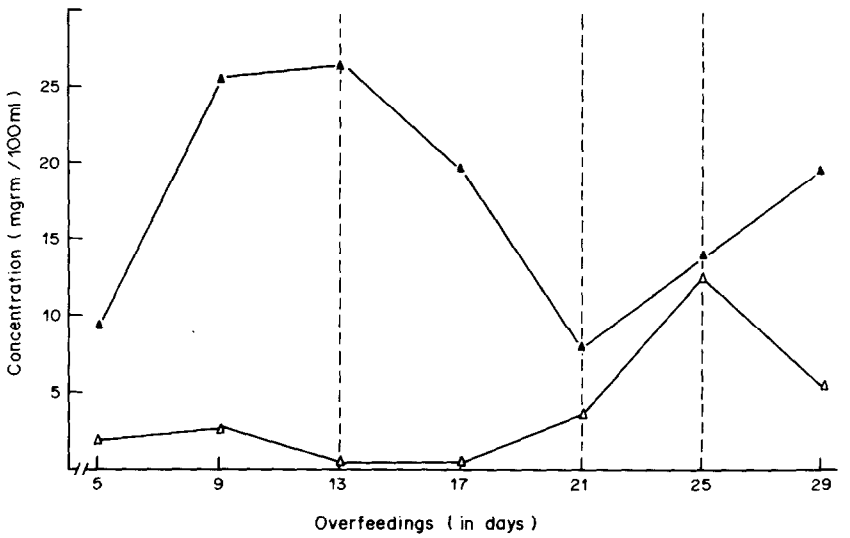

Fig. 1. Experiment IVA. Concentrations of urea (△——△) and uric acid (▲——▲) in crowded media throughout the overfeeding experiments.

B. Analysis of uric acid and urea in pupae and larvae. Table 8 shows the mean concentrations per larva or pupa of urea and uric acid. The concentration of uric acid in larvae is kept more or less constant throughout the different overfeedings, though as has been seen in the preceding section this concentration varies widely in the culture media. On the other hand, in pupae the uric acid content increases with time, as can be expected taking into account the progressive maturity of pupae without excretion. As regards urea, the concentration per larva and per pupa is kept rather constant and low. As a result the concentration of uric acid increases in the pupa while the concentration of urea remains constant. Therefore, at least we may say that metabolism in the pupae does not lead to the production of urea, although this product appears in larvae.

DISCUSSION

The study of larval stop began when this phenomenon was detected by Mènsua and Moya (1983) in an approach to a controlled study of competition situations, disrupting them by means of overfeeding. This larval stop is not a diapause, but a quiescent phenomenon common in competition situations in insects.

The experiments dealing with conditioned media contribute to clarify, up to a point, the influence of excretory products on survival and mean developmental time. Media wasted for 13 days are able to support the development of second-instar larvae (4- and 6-day old larvae) until the third instar or pupal stage and until pupa or adult stage for larvae aged 8 days or more. An important question which arises here is the development stage susceptible to the hypothetical intoxication. The results indicate a slight development delay even in 10-day old larvae in the conditioned media. These larvae able to pupate in the absence of food, suffer a delay when placed in polluted media. If these larvae can pupate during starvation, it is because no limiting factors are needed. Nevertheless, they are delayed in their development, which can only be explained in terms of intoxication. Larvae older than 10 days do not suffer any delay in their development. Perhaps from the 12th day on, larvae have passed a physiological point which must lead to pupation.

The following experiment can help us to distinguish between intoxication and limiting factor(s) in relation to the larval stop. In Table 9 survival and mean developmental time are shown. These results have been obtained in experiments at a different temperature (25 ± 1°C) and with overfeeding at 8th, 10th, 12th, 14th and 16th day. The overfeeding media was composed of agar, sucrose, salts and distilled water (minimum medium) in the same quantities as

Table 8. Experiment IVB—Analysis of urea and uric acid per larva and pupa just before each overfeeding*

Overfeeding (days)	Urea (mg/100 ml)		Uric acid (mg/100 ml)	
	Larva	Pupa	Larva	Pupa
9	1.05 ± 0.27	—	5.16 ± 0.43	—
13	1.18 ± 0.16	—	3.81 ± 0.63	—
17	1.52 ± 0.10	2.37 ± 0.46	2.25 ± 0.22	8.85 ± 0.42
21	1.51 ± 0.31	1.51 ± 0.30	2.37 ± 0.29	6.92 ± 0.27
25	1.74 ± 0.33	2.11 ± 0.28	3.41 ± 0.73	13.71 ± 0.69
29	1.42 ± 0.59	2.07 ± 0.48	3.18 ± 0.14	16.68 ± 3.15

*Each replica was made over 20 larvae or pupae. The total concentration determined in this way was divided into 20.

Table 9. Mean survival (S) and mean developmental time (MDT) with standard errors in inner, outer and total populations throughout overfeeding in minimum medium of crowded cultures at 25°C

	Inner		Outer		Total
Overfeeding	S	MDT	S	MDT*	S
Control 5 ml	—	—	59.0 ± 0.6	12.78 ± 0.13	59.0 ± 0.6
8	7.7 ± 1.9	17.51 ± 0.58	13.5 ± 2.2	15.05 ± 0.22	21.2 ± 2.7
10	8.0 ± 0.9	16.00 ± 0.56	14.3 ± 2.1	16.98 ± 0.42	22.3 ± 2.6
12	12.5 ± 2.2	15.05 ± 0.19	9.2 ± 1.2	18.29 ± 0.12	21.7 ± 2.6
14	15.5 ± 1.9	15.50 ± 0.26	11.8 ± 0.8	20.97 ± 0.18	27.3 ± 1.4
16	17.7 ± 1.8	15.47 ± 0.16	3.8 ± 1.4	22.52 ± 0.40	21.5 ± 1.9
Control 0.5 ml	15.2 ± 1.5	15.93 ± 0.38	—	—	15.2 ± 1.5

*Regression of MDT over overfeeding (outer populations): a = 7.40, b = 0.95 and $R^2 = 0.99$.

in Lewis' medium. These results must be compared with those of Ménsua and Moya (1983). In this case, overfeeding contributes only to collect stopped larvae. The total survival is the same throughout overfeeding, different from that obtained when overfeeding is done in Lewis' medium. The larvae recoverd by overfeeding may or may not have obtained their nutrient requirements, owing to the limiting amount of food available in the competition medium. The larvae that have not obtained their nutrient necessities may complete them during overfeeding in Lewis' medium [better total survival in the early overfeedings than in the last ones. See Ménsua and Moya (1983)]. This is not the case in overfeeding in minimum medium (the same total survival throughout overfeeding). In spite of the different survival in both experiments, larval stop is detected in both cases. As a consequence we must conclude that scarcity of some nutrients is not the direct origin of larval stop. These results contribute in this way to enhance the intoxication hypothesis.

The results obtained by Palabost (1973) show that some unknown products excreted by *rosy* and *scarlet* larvae inhibit their own development. Moreover hypoxanthine is mentioned as a possible cause of this inhibition, but the results obtained in tests showed to be negative. In this sense, the results of our work show, on the contrary, that urea as well as uric acid account fairly for the results obtained in overcrowded conditions. The reason for choosing these two products is the following: both are waste products in the nitrogen metabolism, but in different degrees depending on the kind of animals. *Drosophila* and most of the terrestrial insects are uricotelic. Nevertheless the enzymatic relationship between these two molecules (through the action of the uricase) was an encouragement to carry on the experiment with both products. The effect of urea and uric acid is the following: both produce a development delay at high doses which may account for the results observed in crowded conditions. Both reduce survival, more so in the case of urea. At a high dose they also produce larval stop, as shown by the results of the overfeeding experiments in non-crowded conditions with urea or uric acid. These results show a larval stop in both cases though the time of delay is modified in both directions: urea increases the time of development in the last overfeedings compared to crowded conditons, but uric acid at the dose employed has a smaller effect than the crowded conditions on the developmental time. The differences found in respect to the development time can be explained in the following ways: (i) In the competition media, urea as well as uric acid may be present (as is demonstrated in the results part IV). The effect detected is thus the average of both products (ii) owing to its low concentration, presumably the contribution of urea to the results is reduced compared with uric acid (iii) the uncrowded situation would favour a faster metabolic rate which could reduce the period of detoxification which would operate in the overfeeding but uric-acid-free media (iv) diverse factors may also be acting as differences in the type of vial [small vial in crowded conditions compared with large vial in tubes supplemented with urea or uric acid] (v) the possible scarcity of some products in crowded conditions may also account for the differences between the two situations (crowded and non-crowded conditions with urea or uric acid).

The quantitative analysis of the media allow us to divide development of crowded cultures into different periods, each explicable in terms of a process of complex dynamics where the inner and outer larvae densities must be playing the main role. In the first period from 0 to 13 days (Fig. 1), larvae develop and progressively invade the culture media leaving uric acid as the main excretory product. An important feature to be taken into account is the position of the larvae, which form a circular crown a few millimeters wide. The urea excreted may be produced by uricase action which has been demonstrated to be present in other Diptera (Thruszkowski and Chajkinown, 1935). Once larvae have invaded all the media they are forced to eat their own excretory products: in this phase ingestion is greater than excretion, which can lead to two possible fates: (i) an energetic expense owing to an increased metabolic rate employed in the degradation of this uric acid excess; and (ii) a conversion of uric acid to intermediates of nitrogen metabolism different from urea. As a consequence of this, larval stop is produced and perhaps larvae in these conditions cannot pupate unless they excrete the excess of nitrogenous compounds ingested with the food. This excretion would occur while eating non-polluted food in the overfeeding media or about 21–25th day in the culture media. Pupation in the latter is related to an increase in uric acid and urea in the media. The abnormal increase in urea must be due to an increase in uricase activity which would contribute to the faster elimination of uric acid. In the last phase, when there are almost no larvae in the media, uric acid and urea probably will be decomposed into ammonia by the yeasts present in the media. The various results presented here reveal that

natural waste products like urea and uric acid might explain the phenomena usually observed in competition conditions: development delay and lower viability as well as the stop in larval development, only detected by overfeeding (Moya and Ménsua, 1983c).

From the amount of uric acid and urea detected in larvae, it may be concluded that stopped larvae keep a constant level of these products internally, though the level of these in the media vary. Thus, the larvae must be using energy to degrade the excess of uric acid that they have ingested in order to keep a constant level. As regard the possible action of these metabolites which might account for the observed responses, it remains unknown. In some or other way they must affect the ecdysone and juvenile hormone level which is regulating all the development.

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