Botella, L.M. and J.L.Mensua. University of Valencia, Espana. Determination of the urea and uric acid content in D.melanogaster bred in non-crowded conditions.

In the course of the studies about larval development in crowded cultures of D.melanogaster, larval stop was detected by Mensua & Moya (1983), as a phenomenon which takes place at third instar of larval development. Further studies showed that urea (Botella et al. 1983a), as well as uric acid (Botella et al. 1983b) might account for the results usually observed in competition cultures. Both products have been shown to be present in D.melanogaster culture media. In order to investigate the relative quantities of these products in the bodies of stopped larvae, we have first studied the level of both products from 3rd instar on in larvae bred in non-crowded conditions. The method employed was as follows: seventy newly hatched larvae were seeded in 5 ml. of Lewis' medium. The culture was kept at 18.5±1°C in a thermoregulated room, at 70% relative humidity. At different days from the seeding day, larvae or pupae were extracted, washed in distilled water, dried on filter paper and weighed. Afterwards groups of ten larvae or pupae or adults (depending on the day of culture) were homogenized in 95 µl of Sodium Acetate 0.1 M, the homogenates were centrifuged at 4000 rpm for 5 minutes in a Beckman centrifuge and the supernatants recovered were subjected to quantitative analysis for determination of urea and uric acid contents following the method proposed by Lemar & Bootzin (1957) for urea and by Collins et al. (1959) for uric acid. A total of five replicas were made. The results obtained are shown in Table 1. As can be seen the content of uric acid in larvae of third instar decreases before pupation because in one

Table 1. Urea and uric acid levels at different stages of D.melanogaster development.

Days from the seeding day	Mean weight (mgrm./per individual)	Estimation of urea concentration* (mgrm./100ml./ mgrm.fresh weight)	Estimation of uric acid concentration* (mgrm./100ml./ mgrm.fresh weight
12 (3rd instar larvae)	1.70 ± 0.05	1.0 ± 0.1	5.3 ± 0.3
14 (1 day-old pupae)	1.40 ± 0.05	0.3 ± 0.1	3.7 ± 0.4
20 (7 day-old pupae)	1.60 ± 0.05	1.7 ± 0.5	4.3 ± 0.3
23 (excretion)	-	1.2 ± 0.7	17.0 ± 2.0
24 (adult)	1.00 ± 0.03	3.5 ± 0.7	17.0 ± 1.0

* These data were obtained with the following expression:

$$\text{Concentration} = \frac{(\text{Absorbance of each sample/standard absorbance}) \times \text{Standard concentration}}{\text{Mean fresh weight}}$$

The estimated concentrations will result from multiplying each result by the dilution factor. If we consider that each larva has an inner content about 1 microliter, then this factor would be approximately 1/10.

day old pupae the content is greatly diminished(5.3 in 3rd instar and 3.7 in one day old pupae). The figures which appear in Table 1 have been multiplied by a dilution factor (about 10) in order to obtain the quantitative values (see footnote in Table 1). During pupal stage a progressive accumulation of uric acid must occur owing to the fact that pupae cannot excrete into the media (uric acid content increases from 3.7 in one day old pupae to 4.3 in seven day-old pupae). For the last two phases analysis, mature pupae (ten day old) were incubated at 25±1°C for 20 hours allowing in this way the total emergence of all the adults. The analysis of the first excretion made by the recently emerged flies shows a high level of uric acid as can be expected after the completion of pupal stage in which external excretion does not occur. In the adult between 3 hours and 20 hours old, the concentration of uric acid is also high, which may be the result of a faster metabolic rate in the adult stage and at 25°C.

The urea content is kept at low but detectable levels throughout development, as a result we must admit that uricase acts in D.melanogaster breaking uric acid into urea.

References: Botella, L.M., A.Moya & J.L.Mensua 1983a, DIS 59:23-24; Botella, L.M., C.Gonzalez & J.L.Mensua 1983b, EDRC, Cambridge; Collins, P.F., H.Diehl & G.F.Smith 1959, Analytical Chemistry 31:1862-1867; Lemar, R.L. & D.Bootzin 1957, Analytical Chemistry 29:1233-1234; Mensua, J.L. & A.Moya 1983, Heredity 51:347-352.

Botella, L.M., A.Moya and J.L.Mensua. University of Valencia, Espana. Effect of butyrate on the development of D.melanogaster.

In the course of larval competition studies, larval stop in development was detected by Mensua & Moya (1983) by means of the overfeeding technique (Moya & Mensua 1983). In an attempt to find out the possible origin of this stop produced in crowded cultures, some natural waste products were assayed for their ability to reproduce the larval arrest in non-competitive conditions. Urea was first shown to delay larval development (Botella et al. 1983a), and this result was also confirmed for uric acid (main waste product of the Nitrogen metabolism in Insects).

Moreover both urea and uric acid were shown to be able to mimic the larval stop detected in overcrowded conditions (Botella et al. 1983b). Following the series of experiments with products which might reasonably reproduce the above results, to go more deeply into the mechanism of larval stop, Sodium Butyrate was assayed. The effect of Sodium Butyrate was assayed by adding this product in different concentrations (25 mM, 50 mM, 100 mM and 200 mM to Lewis' medium). Seventy larvae of an isogenic Oregon-R strain were seeded in 5 ml. of Lewis' medium (non-crowded cultures). The temperature was kept at 25±1°C. A total of five replicae were made at each dose, and a control of Lewis' medium without Sodium Butyrate was made. Table 1. shows the effects of Sodium Butyrate on survival and development time. As can be seen, there is an increase in development time with the Butyrate concentrations and survival decreases greatly from 0 to 200 mM.

Table 1. Effect of Sodium Butyrate over Mean Survival (S) and Mean Development Time (MDT).

Dose	S	MDT
0 (control)	56.6±2.7	13.51±0.14
25 mM	51.4±1.5	12.50±0.15
50 mM	34.2±4.3	14.75±0.49
100 mM	28.2±2.7	14.34±0.13
200 mM	6.6±1.4	17.21±0.28

Table 2. Mean survival (S) and Mean Development Time (MDT) in inner and outer population throughout overfeedings in crowded conditions (control) and for non-competitive media supplemented with 50 mM and 100 mM of Sodium Butyrate.

Overfeedings	S			MDT					
				Control		50 mM		100 mM	
	Control	50 mM	100 mM	Inner	Outer*	Inner	Outer**	Inner	Outer***
Control 5 ml.	61.8±0.8	54.0±1.4	31.4±2.9	-	13.7±0.1	-	15.4±0.1	-	18.2±0.1
8	61.0±2.2	45.0±5.1	39.8±3.6	14.5±0.5	16.5±0.2	15.2±0.2	15.3±0.1	-	17.9±0.1
10	50.8±4.8	52.8±2.5	27.2±1.0	14.4±0.2	18.4±0.1	14.7±0.1	16.3±0.2	-	18.7±0.1
12	54.5±2.7	51.6±0.4	35.4±1.6	15.2±0.1	20.5±0.1	15.9±0.1	18.1±0.1	16.8±0.3	19.2±0.1
14	36.2±4.2	52.2±1.9	37.6±1.1	15.6±0.5	22.4±0.3	15.7±0.1	21.0±1.0	18.1±0.1	20.9±0.1
16	34.2±2.0	55.6±2.3	29.6±1.1	14.1±0.1	25.0	16.3±0.1	-	18.8±0.1	23.1±0.9
0.5ml Control	19.8±2.2	-	-	17.3±0.2	-	-	-	-	-

* 1=7.97; b=1.05; R^2=0.998. ** a=7.31; b=0.94; R^2=0.970. *** a=12.42; b=0.63; $R^2$0.960.