that drug metabolism in isolated hepatocytes from diabetic rats was inhibited at shorter durations of diabetes; such an effect was attributed to some transient effect of SZ[18]. It was reported that focal hepatic necrosis in rats could be observed 2-36 h after the injection of SZ, but no other evidence of gross direct toxic damage in liver[23].

In conclusion, as impaired hepatobiliary function was not correlated with hyperglycemia we can assume that it may be a consequence of a direct effect of SZ rather than of the induced diabetic state. Such an effect must be considered in studies on hepatic metabolic disorders in experimental diabetes caused by SZ.

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Oviposition rhythm of individual *Drosophila pseudoobscura*

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Summary. *D. pseudoobscura* oviposits mostly during the day with some oviposition at night. Oviposition varies from tight clustering to loose scattering from different individuals. Daily oviposition ranges from good to poor to no eggs over 24 h. Although this species was an excellent model for the eclosion rhythm it does not serve as a good model for continued study of the oviposition rhythm under present experimental conditions.

Eclosion rhythm studies of *Drosophila pseudoobscura*[2,3] are now part of the historical support for our understanding of the 'biological clock'. However, *D. melanogaster* is by far the more popular choice of the two species when using physiological and genetic analysis[4-6] of the way the clock works. Another rhythm, the oviposition rhythm, has been investigated in *D. melanogaster*[7,8] and it appears to be promising for study in greater depth. Because of its past role in clock studies it was thought prudent to investigate the oviposition rhythm of *D. pseudoobscura* and compare it to the oviposition rhythm of *D. melanogaster*. Oviposition information about *D. pseudoobscura* is sparse[9,10].

My report will show that the oviposition rhythm of individually-housed *D. pseudoobscura* is greatly different from that of *D. melanogaster*. I also report an improvement on the method previously used[8].

Methods. A stock culture of wild-type *D. pseudoobscura* was generously supplied by C.S. Pittendrigh. The same apparatus, lighting conditions, housing, and culture conditions previously described[8] were adapted for the present study. A conveyor belt moved a food tray 2.54 cm per h while individually housed flies descended from a fixed chamber to eat and oviposit in the food. The food was pressed into channels carved into a plastic tray.

During the study, an improved method was developed, as follows. Tightly woven dark blue polyester cloth was cut to fit the surface of the food tray. The cloth was moistened with a boiled solution consisting of 10 g soluble starch, 20 ml corn syrup (Karo, CPC International Inc. Englewood Cliffs, N.J.) in 1 l of H_2O. When stretched on the tray the moist cloth was painted with a yeast (Red Star, Milwaukee, WI) suspension (10 g/100 ml). On the conveyor belt, 25 individually housed flies convert the food tray into an egg tray. The process of making ovigraphs from the egg tray was the same as reported earlier[8]. The old method was used with *D. pseudoobscura* several times before the newer cloth method was adopted. Only results using the cloth method will be reported, along with 1 typical result.

Results. The cloth food tray method is an improvement over the channel method for feeding and oviposition. Eggs on the dark cloth are clearly visible without optics, thus giving an immediate assessment of an experimental run when trays are changed each day. Search and recording time is greatly reduced because eggs are no longer embedded in the food in the channels but are on the cloth surface. A dry egg cloth could be saved if necessary for a voucher specimen of an experiment, because dry eggs

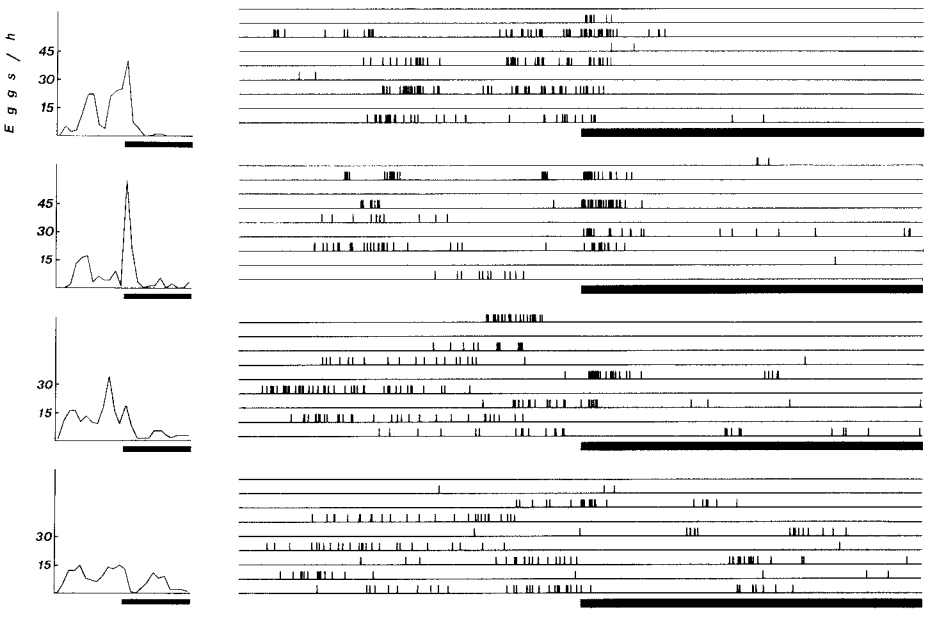

Figure 1. Ovigraphs and summary line graphs of oviposition from 4 selected flies over a 9-day period. Black bars are the dark period of a 12L:12D lighting regimen. Each horizontal line represents one 24-h-day, each vertical stroke represents 1 egg.

adhere to the starched cloth and will not hatch. Otherwise the cloth is washed and reused.

Of the 25 flies tested in one of the experiments were selected to show variability of oviposition within this species (fig. 1). The ovigraphs and line graph summaries indicate a general daytime oviposition with a sharp peak near or at dusk for 2 of the 4 examples. Fly 1 (top graph) shows little or no oviposition after the first hour of the night but in fly 4 there is a mode at mid-dark. Comparison of daily ovigraphs shows that fly 1 and fly 2 have a generalized bimodal oviposition but each expresses the oviposition differently. Fly 1 oviposits mostly in the day but fly 2 divides its oviposition: one mode at about mid day and its 2nd consolidated mode right after dusk. Both flies show a tighter clustering of oviposition than do flies 3 and 4.

Daily oviposition is not a consistent trait. For example, fly 2 missed its oviposition on 2 nights (days 3 and 5). Other flies may have little or no production on certain days or show good production several days in a row (fig. 2). The skipped days are not an artifact of the method because skipped days of different flies do not coincide.

In the daily profile shown in figure 1 the first 4 (A–D) represent oviposition seen in figure 1. No fly shows a pattern resembling another. The clustering or loose pattern of oviposition seen in flies 2 and 4 respectively was also typical of flies fed vinegar food in channels[8], indicating that this food or the presence of the channels had no apparent influence on *D. pseudoobscura* oviposition. *D. melanogaster* will oviposit during the day and at night with vinegar food but generally only early in the night on control food[8].

Discussion. *D. pseudoobscura* does have an oviposition rhythm but it appears not to be as good a model as *D. melanogaster* using present methods. The skipping of days, the daily production variability and the variable loose or consolidated oviposition in different individuals make a simple approach to a further understanding of the rhythm in this species difficult.

When individual flies are studied, the variability is easily noticed and could perhaps serve in genetic selection. Other selection studies[9,10] make use of populations tested in various environmental conditions. The 4 examples of oviposition selected from 25 flies in one of several experiments were only obvious differences in a subtle continuum. Yet

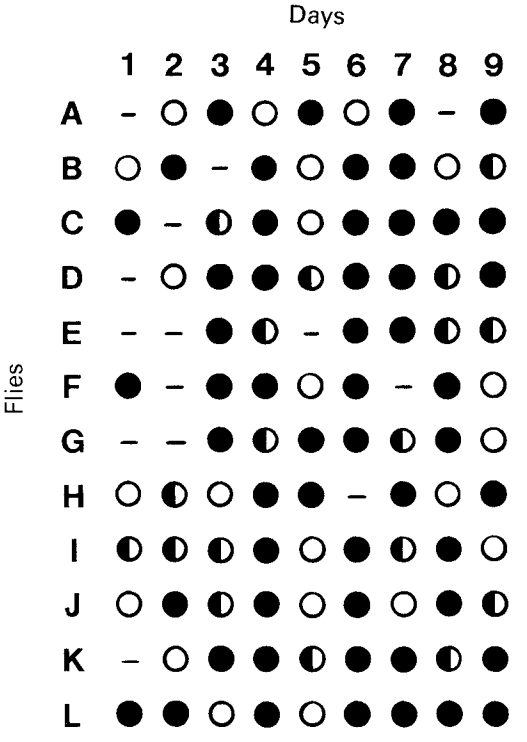

Figure 2. Example of the variability of daily egg production for 9 days. Flies A, B, C, D represent the same egg production as is shown in figure 1. Symbols: dash, no eggs; open circle, less than 10; half circle, 10–19; full circle 20 or more eggs per day. Note the lack of consistency from day to day and between individuals. The highest producer was fly J with 225 eggs, the lowest was E with 125 eggs in the 9-day period.

the general conclusion is that *D. pseudoobscura* oviposits with 2 modes mostly during the day but some individuals also have an additional oviposition mode at night.

Eclosion in this species has a single mode with a phase angle about 3 h after dawn[2,3]. The 2 or 3 modes of oviposition in *D. pseudoobscura* suggest a mechanism other than one controlled by a single pacemaker coupled to the eclosion rhythm.

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The effect of glutamate, cysteate and related amino acids on insect muscle

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Summary. Application of Glu, $CySO_2$ or $CySO_3$ to blowfly larvae caused paralysis and increased the membrane potential of larval muscle. The contents of Glu and $CySO_3$ in larval muscle containing motor nerve terminals were markedly decreased after the perfusion of high K^+ saline solution.

It is considered that glutamate (Glu) and aspartate (Asp) function as transmitters in the insect neuromuscular junction and the mammalian brain[1,2]. Among the amino acids, Glu is analogous to Asp and $CySO_3$ (cysteate) in chemical structure and molecular size. Crawford and McBurney[3] analyzed the power spectrum of postsynaptic noise caused by the application of Glu, Asp and $CySO_3$ at the insect neuromuscular junction. It is interesting to note that Tau (taurine), a metabolite of $CySO_3$, has recently been found to be localized mainly in the mammalian liver and brain, and to have a physiological function in the central nervous system[4]. In the insect, it has been shown that a high concentration of Tau is present in insect flight muscle[5]. However, it is not clear whether Tau, Glu and $CySO_3$ function as neurotransmitters in the insect neuromuscular system or not.

In this study, we investigated how Tau, Glu, and $CySO_3$ affect the neuromuscular system of larvae of the blowfly, *Aldrichina grahami*.

Materials and methods. Blowflies, *Aldrichina grahami*, were reared aseptically on semi-synthetic diets at 25 °C as described previously[6]. The amino acids were injected through the spiracular sclerite of the anal side of 4- or 6-day-old larvae. The larval resting time after the injection was measured at 25 °C by phototaxis.

Isolated hemolymph and muscle containing nerve terminals were homogenized in 10 vols of 75% ethanol with a glass-glass homogenizer, and centrifuged at 10,000 g for 10 min. The supernatant was evaporated and delipidized with chloroform/H_2O (2:1). The water layer was adjusted to a pH of 2.0 with HCl. This acidic solution was chromatographed on Dowex 50W-X8 (100–200 mesh, Cl^--form, 1.0 × 5.0 cm). The elution was performed with 3 vols of 0.01 N HCl and 1 vol of H_2O, continuously. These eluates were referred to as the washing fraction. Finally, the column was eluted with 3 vols of 3 M NH_4OH. The ammonium eluate was referred to as the adsorbed fraction. Both the washing and adsorbed fractions were dried with an evaporator and analyzed with a high-performance amino acid analyzer which is equipped with an IEX 215SC column (Toyosoda Co. Ltd., Tokyo, Japan), a visual detector (570 nm) and an integrator (Waters Associates, model 730). The analyzing buffer used was 0.2 M citrate buffer (pH 2.2). Details of the analytical techniques will be published elswhere.

The 4- or 6-day-old larvae were dissected, the viscera and central nervous system removed, and the muscle containing motor nerve terminals and cuticle was perfused with saline solution (172 mM NaCl, 3.3 mM KCl, 1.0 mM $CaCl_2$, 0.7 mM NaH_2PO_4, 20 mM glucose and 10 mM HEPES, pH 7.2) for 30 min before any recordings were made. Intracellular recordings from muscle were made using 3M KCl-filled glass microelectrodes with a resistance of 5–15 mohm. The recordings were monitored using standard procedure.

The isolated muscle preparations were perfused with the normal saline solution mentioned above 30 min before the perfusion of high K^+ saline solution (172 mM KCl, 3.3 mM NaCl, with the other constituents the same as normal saline solution). The perfused muscle preparations were treated by the above-mentioned method.

Results and discussion. The application of Glu, $CySO_2$ (cysteine sulfinate) or $CySO_3$ to the larval body cavity caused marked paralysis, as shown in the figure. When Glu

Table 1. Amino acid contents of larval muscle after high K^+ stimulation and hemolymph

	Muscle (nmole/mg wet weight)				Hemolymph (nmole/insect)	
	4-day-old		6-day-old			
	Control	High-K^+	Control	High-K^+	4-day-old	6-day-old
Taurine	5.87 ± 0.94	6.67 ± 0.94	5.84 ± 0.38	6.51 ± 0.65	25.1 ± 2.4	67.7 ± 2.6
Aspartate	0.21 ± 0.08	0.16 ± 0.06	0.25 ± 0.07	0.16 ± 0.02	22.0 ± 1.1	3.9
Glutamate	0.50 ± 0.03	0.38 ± 0.05	1.58 ± 0.64	0.98 ± 0.27	170.0 ± 9.1	41.6 ± 6.3
Glycine	0.83 ± 0.06	1.24 ± 0.16	0.61 ± 0.05	0.93 ± 0.05	207.5 ± 23.6	49.2 ± 3.6
Alanine	5.58 ± 0.83	8.29 ± 2.08	8.20 ± 1.68	11.59 ± 2.19	307.0 ± 40.3	232.2 ± 12.0
Cysteate	–	–	0.02	–	0	0

Each value represents the mean of 3 determinations ± SD.