$CySO_2$ or $CySO_3$. It has been reported that Asp is a possible transmitter in the mammalian visual cortex[4] and in the muscle of *Musca domestica*[2]. Unfortunately, we could not provide further evidence that Asp plays a role as a transmitter in the larval neuromuscular system.

Consequently, we suggest that Glu, $CySO_2$ and $CySO_3$ may be transmitters in the neuromuscular system of the larva of the blowfly, *Aldrichina grahami*. Further investigation is needed using a specific inhibitor to determine the action of these amino acids on the neuromuscular system.

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Effect of fighting on the hemogram in an insect *Schizodactylus monstrosus* Drury (Orthoptera, Schizodactylidae)

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Summary. Changes were seen in total hemocyte count/mm^3 blood, in different categories of hemocytes and in blood volume during inter-male fighting in *Schizodactylus monstrosus*. Total hemocyte count showed a steady increase up to the end of fighting though the trend was evident 10 min after the beginning of the fighting. The number of granular hemocyte and spherule cells increased during the first 10 min of fighting, whereas the number of adipohemocytes began to increase after 10 min. A slight increase in blood volume was noted during the early periods of fighting.

In insects, the regulation of metabolic processes during energy stresses has been reviewed by Bailey[2]. Attention has mostly been paid to the variation of sugar, lipid and free amino acid levels in the hemolymph, fat body and muscles in relation to different energy stress situations[3-5]. Alterations in the hemogram as the index of various physiological conditions have also been studied[6-9]. Storage of different nutrients like carbohydrate, lipid and amino acids by the hemocytes and their role in maintaining the normal nutrient balance during different stress conditions are well documented[10-12].

Schizodactylus monstrosus Drury (Orthoptera, Schizodactylidae), a nocturnal carnivorous insect, shows intraspecific aggressiveness. In spite of extensive studies of different aspects of insect hemocytes, no attempt has yet been taken to report any variation in the hemogram during intraspecific fighting. The present investigation attempts to report the variation in total number of circulating hemocytes/mm^3 blood, the number of different circulating cell types and blood volume during different time intervals of fighting. The existence of sessile hemocytes in this insect has already been reported[13]. The present communication also deals with the role of sessile hemocytes during fighting stress.

After collection, insects were kept separately in moist sand jars (90% relative humidity). Cockroach nymphs were supplied as food on alternate days. 7-day-old adult males (obtained by rearing the last instar nymphs in the laboratory) were used in this experiment.

Fighting was initiated in the laboratory between 2 adults fed under similar conditions (8 h after feeding). After initiation it continued actively up to 20–23 min, after which the aggressiveness gradually ceased. Thus, to show the sequence of changes in the hemogram, all estimations were made every 5 min up to 20 min from the commence of fight.

Hemolymph samples were collected by amputating one of

Table 1. Total hemocyte count (cells/mm^3 blood) in control and heat-treated *S. monstrosus* in relation to different time intervals of fighting. Values are mean ± SE of 7 replications

	Time intervals of fighting				
	0 min	5 min	10 min	15 min	20 min
Control	16,400 ± 320	17,000 ± 280	20,200 ± 289	28,050 ± 312	30,975 ± 276
Heat-treated	22,800 ± 316*	23,900 ± 281*	23,100 ± 311	29,080 ± 328	31,650 ± 319
% differences	39.02	40.58	14.35	3.67	2.17

* $p < 0.1$ in comparison to control.

the hind-legs. For total hemocyte count (THC), hemolymph was allowed to fill the Neubauer hemocytometer chambers by capillary action about 5 sec after leg amputation. Differential hemocyte count (DHC) was made by staining air dried blood films with Leishman's stain. Cell size, position of the nucleus, nature of cytoplasmic inclusions and staining reactions were used as the main criteria for identification of hemocyte types.

To evaluate the role of sessile hemocytes during different experimental periods, THC and DHC were made from heat fixed insects after subjecting them to 50 °C for 5 min.

For the estimation of hemolymph volume, insects were weighed and injected with 1 µl of 2% Congo red dye, which was allowed to circulate for 5 min. 1 µl of the dyed blood was drawn out and diluted to 1 ml with insect saline. Transmission readings of this dyed blood were compared with that of undyed blood serving as control. Blood volume was estimated by plotting the data using Lee's equation[14].

Total sugar in the hemolymph was determined following the anthrone method of Roe[15] using glucose as the standard. Total lipid was estimated by the vanillin method of Goldworthy et al.[16]. Free amino acids were estimated following the spectrophotometric method of Rosen[17]. For each experiment, a single specimen was used.

At the initiation of fighting THC was $16{,}400 \pm 320/mm^3$ blood. The value showed an 85% increase after 20 min of continuous fighting; the rate of increase was singificant after 10 min of fighting (table 1). Hemocyte categories included prohemocytes, plasmatocytes, granular-hemocytes, adipohemocytes and spherule cells[18]. They formed 20%, 15%, 43%, 18% and 4% of the hemocyte population respectively at the initiation of fighting (table 2). The granular hemocytes and spherule cells were found to increase in number up to 10 min of fighting, while the number of adipohemocytes increased after 10 min of fighting (table 2). Hemolymph volume at the initiation of fighting was 260 ± 0.8 µl/insect; after 10 min, the volume was 264 ± 0.6 µl/insect, after which it showed a negligible increase (only 1 ± 0.03 µl/insect).

Temperature stress resulted in 40.58–39.02% increased THC after 5 min fighting in comparison with normal insects ($p < 0.1$). At the later time-points it showed only 2.17–14.35% increase compared with normal insects during the same experimental period (table 1). After 5 min or more of fighting prohemocyte and plasmatocyte numbers decreased while the number of granular hemocytes showed an increase in the heat-treated insects compared with normal insects during the same time-intervals of fighting. Adipohemocytes exhibited an increased number up to 15 min of continuous fighting in heat-treated insects (table 2).

Total sugar and lipid levels exhibited declining trends showing 9.64% and 7.66% respectively up to 10 min fighting, followed by 9.7% and 34.02% increase after 15 min. Both concentrations, however, showed a total drop of 7.07% and 64.08% respectively after 20 min of continuous fighting. The level of free amino acids showed a gradual decline (up to 51.46%) after 20 min of fighting (table 3).

Alterations in the number of hemocytes in relation to different time intervals of fighting demonstrate their strong response during such physiological stress conditions. Usually, a considerable proportion of the total hemocytes remain adhering to tissue surfaces or stored in temporary reservoirs; these are the 'sessile hemocytes'[6,7]. During any sort of physiological stress situation, these hemocytes are released into the circulation to maintain the homeostatic balance of circulatory hemocytes[13,19]. Temperature stress causes release of these hemocytes into the circulation. Thus the difference in hemocyte counts in normal and heat-treated insects gives an idea of the number of sessile hemocytes in this insect. Data in table 1 show that during the early periods of fighting the number of sessile hemocytes was considerably higher than in later periods, which indicates that such intraspecific aggressiveness accompanied by vigorous exercise is also an external factor that causes release of sessile hemocytes into the circulation.

During the early periods, a slight increase of blood volume seems to be related to the decrease in water content of gut, fat body and muscles in response to such vigorous exercise (personal observation). This insignificant increase in blood volume accompanied by a greater rate of influx of sessile hemocytes into the circulation resulted in a moderately increased THC during the early periods of fighting compared with the latter periods, which might be attributed to a higher rate of release of sessile hemocytes into the circulation.

Histochemical and biochemical analyses of the cytoplasmic inclusions of different hemocytes show that they store various nutrients, which are thought to be utilized during nutritional stresses. During the early periods of fighting, during which there was a depletion of sugar in the hemolymph, the number of granular hemocytes, spherule cells and plasmatocytes showed an apparent increase. These cells are reported to store glycogen, mucopolysaccharides and other PAS positive materials[12,20]. Similarly, during the later periods, the number of adipohemocytes (which store lipids[10,11]) increased corresponding with the depletion of lipid content in the hemolymph. A role of hemocytes in the storage of amino acids is also known[21]. The alterations in the number of circulating hemocytes, maintaining a relation ship with the alteration of nutrient levels in the hemolymph, demonstrates the role of stored foodstuffs in the hemocytes (perhaps by releasing them into the hemolymph) to balance the nutrient level in the hemolymph during periods of acute energy need, since these nutrients are reported to serve as an immediate fuel source during exercise in *S. monstrosus*[22,23].

Table 2. Differential hemocyte count (expressed as %) in *S. monstrosus* under control and heat-treated conditions in relation to different time intervals of fighting. Values are means for 500 cells; each had 6 replicates

Hemocyte types	Control					Heat-treated				
	0	5	10	15	20	0	5	10	15	20
Prohemocytes	20	19	8	7	11	25	17	6	5	6
Plasmatocytes	15	17	19	17	18	18	15	14	11	12
Granular hemocytes	43	49	57	50	31	34	51	60	53	48
Adipo hemocytes	18	9	7	21	35	20	12	9	25	29
Spherule cells	4	6	9	5	5	3	5	11	6	5

Table 3. Total sugar, lipid (mg/100 µl) and free amino acid (µg/100 ml) content of the hemolymph of *S. monstrosus* in relation to different time intervals of fighting. Data are mean ± SE of 9 replications. (For each estimation single specimen was used)

Contents	Time intervals of fighting				
	0 min	5 min	10 min	15 min	20 min
Total sugar	1.14 ± 0.01	1.04 ± 0.01	1.03 ± 0.02	1.13 ± 0.02	1.05 ± 0.01
Total lipid	2.61 ± 0.03	2.56 ± 0.02	2.41 ± 0.03	3.23 ± 0.01	1.16 ± 0.02
Total amino acids	1881 ± 21	1275 ± 19	954 ± 16	840 ± 20	813 ± 23

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Variation in the fatty acid composition of developing seeds of rapeseed

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Summary. A study was made of the variation in the fatty acid composition of the oil in the developing seed of the 'Rafal' cultivar of the *Brassica napus* L. cultivated in central Italy. The oil content reaches its maximum level 60 days after the petals fall. The increase in the percentage of oleic acid is negatively correlated with the palmitic, stearic and linoleic acid content.

The seed of the rapeseed provides a quantity of oil ranging from 40 to 53% of dry matter, depending on the cultivar, and on the region, year and soil in which it is grown[2].

The characteristics of this oil are that it contains eicosenoic and erucic acids which are typical fatty acids of the Cruciferae family to which the rapeseed belongs. This seed is also rich in other fatty acids, namely oleic, linoleic and linolenic acids. In the past, use of the rapeseed in various oleiferous food products was limited because of its high erucic acid content, which sometimes accounts for up to 53% of the total acids[3].

Over the last few years, by means of plant breeding, it has been possible to develop rapeseed cultivars with an extremely low level of eicosenoic acid and which are free from, or contain only a very small amount of erucic acid with, at the same time, a higher oleic acid content[4,5]. Currently there are many varieties of rapeseed with a low erucic acid content which have been grown in very different environments from that of Italy (Canada, Sweden, Germany, France, etc.). Many problems have still to be solved in relation to the varieties, cultivation areas and choice of agronomic techniques.

The scope of this paper is to investigate variations in the fatty acid content, from fertilization to maturity, of the seeds of a cultivar with a zero erucic acid content, cultivated in central Italy. Cultivar Rafal was preferred as it is considered to be well suited to this area[6].

Plant material. Seeds of the 'Rafal' cultivar of the *Brassica napus* L. ssp. *oleifera* DC., winter rape, were sown on October 5 in an open field at S. Apollinare, Perugia (lat. 43 °08′ N; 300 m above sea level). Average temperatures in the coldest month, on flowering and at maturity were 4.4, 8.5 and 18 °C respectively.

Flowering commenced 180 days (April 5) after sowing. Petal fall was taken as a sign that fertilization had taken place. The first samples of developing seeds were taken 10 days after petal fall (APF). Further samples were taken at intervals of 10 days up to the 70th day APF.

Determination of fatty acid. The developing seeds were removed from the plants and extracted with ethyl ether in a Soxhlet extractor and subsequent preparation of fatty acid methyl esters was carried out according to Appelqvist[7]. These samples were analyzed in a Perkin-Elmer 3920 B chromatograph fitted with a hydrogen flame ionization detector and equipped with a 200-cm column packed with 15% w/w diethylene glycol succinate (DEGS) coated on Chromosorb W/HP. The nitrogen flow rate was 20 ml/min and the injector, column and detector temperatures were 250, 200 and 220 °C respectively.

Quantitative estimation of proportions of individual fatty acids was carried out by the integrator Sigma 10 Chromatography Data Station. Determination of absolute amounts of fatty acid was achieved by the use of methyl heptadecanoate as internal standard. The identification of fatty acids was based on the comparison of retention times with those of known reference mixtures.

Samples of other seeds at a similar stage of maturity were taken, for determination of dry weight by oven-drying at 105 °C.

Results. The variation in the percentages of dry weight and oil content during seed development is shown in the figure. The dry weight drops during the first 30 days APF and then

Table 1. Variation in the fatty acid composition of developing seeds of the Rafal cultivar of *Brassica napus* L.

Time after petal fall, days	Fatty acid composition, % 16:0	16:1	18:0	18:1	18:2	18:3	20:1	20:2	22:0	22:1	Other acids
20	11.7	0.6	3.7	40.6	26.2	9.1	0.1	5.0	0.8	0.2	2.0
30	12.7	0.8	3.2	45.6	21.2	11.5	0.2	2.2	0.2		2.4
40	9.1	0.8	3.1	58.3	18.4	7.4	1.2	0.5	0.4		0.8
50	6.1	0.5	1.8	61.5	19.5	8.5	1.2	0.1	0.4		0.4
60	5.5	0.5	1.5	62.0	19.5	8.7	1.2	0.2	0.3		0.6
70	5.7	0.4	1.6	64.0	17.9	8.3	1.2		0.3		0.6