Trehalose in the Flight Muscle of the House Fly, *Musca domestica* L., in Relation to Age

Trehalose has been shown to be the major blood sugar of insects[1-4], which serves as a mobile energy reserve. Indeed, the level of trehalose in the haemolymph of *Phormia regina* has been shown to regulate the rate at which the energy is expended by flight muscles during flight[5]. In the silkworm larva, *Bombyx mori*, trehalose has been suggested as a source of carbon for chitin synthesis during moulting[6]. SACKTOR and WORMSER-SHAVIT[7] most recently have observed that trehalose was utilized at a discontinuous rate during flight by *Phormia regina*, and this suggests the involvement of 2 kinetically different pools of trehalose as being available for flight in this insect.

The present paper describes the age-related changes in the quantity of trehalose in the flight muscles of the male house fly, *Musca domestica* L.

House flies of the NAIDM strain reared and maintained on KLIM®, cane sugar and water at a constant temperature (80 °F) and humidity (50% relative humidity), as described by ROCKSTEIN[8], were used in the present studies. Thoraces from 25 male flies were dissected at each desired age and, after careful removal of the wings and other appendages, were homogenized mechanically in a Potter-Elvehjem homogenizer for 2 min in a known volume of chilled 80% ethanol. The resulting homogenate was centrifuged at 0 °C for 10 min at 4800 rpm, and the supernatant was decanted and evaporated to dryness under reduced pressure. The residue was redissolved in exactly 0.25 ml of distilled water and, when necessary, centrifuged cold for 5 minutes at 2000 rpm. Known quantities (10 μl–15 μl) of this final extract were then deposited on (Whatman No. 1) filter paper chromatograms, the lower ends of which were serrated in order to allow the solvents to drip uniformly. The chromatograms were developed unidimensionally in a descending solvent system, *n*-butanol-ethanol-acetone-water (5:4:3:2 v/v) for about 40 h at room temperature. The chromatograms were then air-dried and dipped in silver nitrate solution to reveal the various sugars, according to the method of TREVELYAN et al.[9]. Sugars present were identified by comparing their relative positions with known carbohydrate compounds run side by side on the same chromatogram. Identification of trehalose was further confirmed by the fact that the spot suspected to be that of trehalose, after being eluted with water and hydrolyzed with 1 *N* sulphuric acid, gave only glucose and that the chromatograms dipped in benzidine-trichloroacetic acid did not show any spot corresponding to that of trehalose revealed by the TREVELYAN silver nitrate method.

For quantitative estimation of trehalose, appropriate areas corresponding to the pure sugar (trehalose) were cut from the developed but unstained chromatograms and were eluted in 4 ml of distilled water. To this was added 8 ml of freshly prepared anthrone reagent (0.2% of anthrone in 95% H_2SO_4) and the mixture was allowed to stand at room temperature for exactly 45 min, after which readings were taken at 620 nm in a Bausch and Lomb spectrophotometer.

The data reported in the Table represent the mean values obtained for 4 generations of flies.

Concentration of trehalose in the flight muscle of the male house fly, *Musca domestica*, reaches a maximum within 4 h after emergence and, by 24 h of adult age, it has decreased to about $^1/_3$ of the maximum. The 24 h level of trehalose remains fairly constant and there is no significant change thereafter in the flight muscle trehalose content as the fly ages. It should be noted that flies emerge with a fairly high concentration of endogenous trehalose, since the 1 h old flies which contain 15–20 μg of trehalose per thorax had not been given any food previously. It is interesting to observe that the most active period in the life of the adult male fly is between a few hours after emergence to 48–72 h of adult age. During this period, the males are seen actively flying in the cage and copulating with the females. It has been reported by ROCKSTEIN and BRANDT[10] that the male house flies start losing their wings by the middle of the first week after emergence and that by the 7th to the 12th day of adult age, most of the males become wingless. Thus, this onset of abrasion (and ultimate loss) of wings in the early stages of adult life occurs within 24–48 h following the pronounced drop in concentration of trehalose in the flight muscle, observed in the present study.

These data are especially interesting, in view of the fact that ROCKSTEIN and BRANDT[10] reported that the Mg-activated ATP-ase activity in the giant mitochondria of the flight muscle of aging house flies reached a peak at the 5th day of adult life, following which it falls precipitously and then more gradually, quite steadily throughout adult life. In the case of the extramitochondrial α-glycerophosphate dehydrogenase, on the other hand, the peak of activity occurs at between 3 and 4 days of adult life, following which there is a rapid fall to about the 8th day and then a gradual, very slow decline from the 8th to the 18th day, which can almost be described

Trehalose content in the flight muscles of the aging male house fly, *Musca domestica* L.

Age	Trehalose content per thorax (in μg)
1 h	20.15
4 h	26.50
8 h	18.11
18 h	15.63
24 h	8.59
48 h	7.37
72 h	11.13
4 days	7.53
5 days	7.51
6 days	8.48
7 days	7.84
8 days	8.50
9 days	6.26
10 days	7.05
12 days	8.41
16 days	4.71

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as a slow leveling-off period[10]. This appears to parallel very closely the data for trehalose reported in the present paper and suggests further that there is a series of biochemical changes which precedes the failure of flight ability, which begins at approximately the 4th to the 5th day and reaches a maximum by the 7th day of adult life and which must follow prior (causative) chemical events and accompany the more readily apparent deteriorative, structural and functional changes in flight ability. Related studies are now underway on the changes in the enzyme trehalase in the flight muscle of the house fly, with age, as well.

Since ROCKSTEIN and BHATNAGAR[11] have observed that high levels of X-irradiation to the early pupal stage of the adult house fly results in enhancement of wing retention at the time of death in both males and females, it would be interesting to determine the effects of such X-irradiation on trehalose content and, therefore, trehalose metabolism, following similar X-irradiation of the pupae. Such studies are also currently being undertaken in this laboratory[12].

Zusammenfassung. Trehalose der Flügelmuskeln männlicher Hausfliegen, *Musca domestica* (1 h bis 16 Tage alt), wurde mit Filtrierpapierchromatographie getrennt und quantitativ kolorimetrisch bestimmt. Die Konzentration der Trehalose in den Flügelmuskeln erreicht ihr Maximum (26,50 µg/Thorax) 4 h nach dem Schlüpfen der Imago. Nach 24 h (Imago) auf $^1/_3$ des Maximums (8,59 µg/Thorax) vermindert, stabilisiert sich der Trehalosegehalt bis zum Lebensende.

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Effects of a Water Soluble Extract of *Robinia Pseudoacacia* Leaves on Uterine Smooth Muscle

Toxicity of the Black Locust (*Robinia pseudoacacia*) leaf has been known for many years[1-3] and several toxic substances from this plant have been identified[4-6]. However, a new activity has been described by this laboratory and that is the stimulation of increased spontaneous activity in rat myometrium.

A decoction was prepared by grinding fresh green leaves, percolating them with boiling water (pH 5–6) for 2 h then filtering through several layers of gauze and finally through Whatman No. 1 filter paper. 1 l of the resultant solution contained the extract of 112 g of green leaves. The decoction was then adjusted to isotonicity by the addition of sodium chloride.

The effect of the solution on the isometric spontaneous pattern of uterine cornu from young, 200–250 g, virgin Sprague-Dawley rats was studied. One end of the uterine cornu was firmly anchored to a warm chamber holder while the free end was suspended from a force-displacement transducer (Model FT .03, Grass Instrument Company) which in turn was connected to a Grass polygraph. Each preparation was immersed in a 50 ml bath containing Tyrode's solution at 37 °C and aerated with 95% O_2 and 5% CO_2. Tension was adjusted to 0.2 g during the non-contracted phase. Following a 30 min equilibration period the minimal stimulatory dose (3 ml of the filtrate) of the solution was added to the bathing medium. Contractual measurements were made at 15, 30 and 45 min and were compared to the zero time readings and to the activity of the contralateral uterine horns suspended in a similar bath treated with saline.

The solution was tested on uteri from 10 dioestrous and 10 oestrous animals. It increased the frequency of contraction of oestrous uteri at 15 and 30 min but not at 45 min relative to zero time observations and at all

Treatment	Time (min)			
	0	15	30	45
	Oestrous			
	Strength of contractions (g)			
Control	2.24	1.14	1.15	1.08
Decoction	1.15	3.73 [b,c]	4.06 [b,d]	4.65 [b,d]
	Frequency of contractions (contractions/min)			
Control	0.94	0.32 [b]	0.32 [b]	0.30 [b]
Decoction	0.70	1.20 [b,d]	1.03 [b,d]	0.90 [d]
	Dioestrous			
	Strength of contractions (g)			
Control	2.53	2.60	2.52	2.36
Decoction	3.24	6.92 [b,d]	7.08 [b,d]	5.79 [b,d]
	Frequency of contractions (contractions/min)			
Control	0.96	0.72 [a]	0.71 [a]	0.64 [b]
Decoction	1.04	1.24 [a,d]	1.28 [a,d]	1.15 [d]

[a] Significantly ($p < 0.05$) different from zero time within the same treatment. [b] Significantly ($p < 0.01$) different from zero time within the same treatment. [c] Significantly ($p < 0.05$) different from non-treated control at the same time period. [d] Significantly ($p < 0.01$) different from non-treated control at the same time period. 1 uterine horn from each of the 10 rats was treated with decoction of Black Locust leaf (6.7 mg/ml) and the other served as an untreated control. Each horn was observed at 4 time periods, thus forming a split plot in time. The measurements recorded at 15, 30 and 45 min were compared with the zero-time observations within both the control and treated uterine horns. Comparisons between the control and treated horn were also made within each time period.