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.

M. ROCKSTEIN and P. N. SRIVASTAVA

Department of Physiology, University of Miami, School of Medicine, Coral Gables (Florida 33134, USA),
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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.

times relative to the contralateral cornu. Significant increases in frequency of contraction of dioestrous uteri were observed similarly to those of the oestrous uteri. It significantly ($p < 0.01$) increased tension in both types of uteri at each of the time intervals. Results from this experiment are summarized in the Table. Preliminary studies indicate that this solution has similar effects on excised human myometrium.

It may be that this oxytocic agent is specific for the smooth muscle of the uterus since it was without effect on 10 samples of rat duodenal tissue. These intestinal segments were treated identically to the uterine strips and doses 10 times greater than those causing uterine stimulation were without effect on the intestinal preparations. Also the sensitivity of vascular smooth muscle to the decoction was tested. The right carotid arteries of 10 rats were cannulated, and blood pressure, pulse pressure and heart rate were monitered on a Grass polygraph. Injections of 1 ml of extract per 100 g of rat via the femoral vein produced no change in any of the measured parameters up to 1 h after the injections.

At the present time the decoction has resisted complete purification, but several observations have been noted with regard to properties of the active agent. It is stable at 100 °C, is not decomposed by light or upon drying, nor is it soluble in the fat solvents (petroleum ether, benzene, diethyl ether, acetone, heptane or methanol). It is not retained on Sephadex G-25 or G-50 but is trapped on an 18 · 1 inch column of G-100 grade Sephadex. Inorganic ion effects have been ruled out as both charring and treatment with acid (pH less than 2) cause loss of activity when the extract is returned to its original conditions[7].

Résumé. Un extrait aqueux de feuilles de *Robinia pseudoacacia* augmente la fréquence et la force de contraction de l'utérus isolé de rat. Cette action semble être spécifique pour le muscle utérin lisse, car l'extrait est sans effet sur des préparations isolées d'intestin et n'agit pas sur la pression artérielle du rat intact.

B. B. PHARRISS[8] and R. L. RUSSELL

Department of Pharmacology, University of Missouri Medical Center, Columbia (Missouri, USA),
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8 Present address: Metabolic Diseases Research, The Upjohn Company, Kalamazoo, Michigan.

Inhibition of Shivering Obtained by Peripheral Stimulation

Afferent input elicited by peripheral stimulation was shown to inhibit several functions of the central nervous system. Inhibitory behaviour with drowsiness was obtained by low rate stimulation of cutaneous nerves[1] and by repetitive isolated electrical pulses to the s.c. tissue[2].

Moreover, autonomic activities may also be inhibited by afferent stimulation, i.e. the inhibition of the skin galvanic reflex induced by faradization of cutaneous nerves[3], and the hypotensive reflex obtained by skin stimulation in the anaesthetized dog with chloralose (in preparation). It seemed probable that other activities of the central nervous system, such as shivering, might be inhibited by this form of stimulation; and, in view of the fact that there is no definite conclusion on the matter[4], this has been investigated.

Methods. 9 mongrel dogs weighing 7–18 kg were anaesthetized with nembutal (33 mg/kg, i.p.). Shivering was detected by the EMG activity registered from the extensor and flexor muscles of 1 leg. The electrodes were applied by visualization of the muscles after incision of the skin and secured by ligature to avoid any mobilization from the implanted place. Thin stainless steel needles or nichrome wire 0.2 mm width isolated except in 10–15 mm of length were utilized as electrodes. 2 electrodes were applied in each muscle separated by a distance of 10 mm. The electrodes were connected to the AC input of a 7 Model Grass Polygraph preamplifier with a time constant of 0.04 sec. The same leg implanted with the EMG electrodes was stimulated with pulses of 0.5 msec duration and 30 c/sec by means of needles introduced in the foot pad of the leg. Usually, the forelegs were preferred to register the EMG activity, but shivering could as well be detected from the hindlegs.

Results. EMG activity was poorly developed during the first hours of the experiments. After the first or second hour, before the appearance of shivering, it was possible to register some tonic EMG activity from the extensors. The definite figure of background EMG activity depended on the position of the leg, although a tonic flexor activity was always undetectable with the sensitivity used. Shivering started about 2–3 h after injection of the anaesthetic and was fully developed by 4–5 h, when signs of lighter anaesthesia level appeared. The rectal temperature was slightly subnormal (0.5–1 °C) at the beginning of shivering and thereafter higher values were recorded. Usually shivering attacks lasted no longer than 30 sec and began by tremor of the head muscles. Shivering activity was more evident during the inspiration. Figure (a) is a typical record where a tonic extensor activity is predominant. It is also shown that modifications occur on the EMG during shivering attack. The beginning of shivering is indicated at the arrow by an abrupt activation of flexor muscles concomitantly with an augmenta-

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