# Quantitative Measurement of Muscle Contractions Associated With the Hindgut and Oviduct of the Stable Fly (Diptera: Muscidae)[1]

B. J. Cook, T. Peterson, and B. Staneart[2]

Veterinary Toxicology and Entomology Research Laboratory, ARS-USDA
Route 5, Box 810, College Station, TX 77840

J. Entomol. Sci. 25(1): 99-105 (January 1990)

**ABSTRACT** A miniature force transducer system was developed to detect and quantitate the contractile events of the hindgut and oviduct of the stable fly. The system readily distinguishes recorded changes in contractile force over a range from 350μg to 35 mg on myographs of these tissue preparations.

**KEY WORDS** Force Transducer, Mechanical action of muscle, Myographs.

The study of insect muscle physiology and pharmacology invariably requires some type of device to record movement. The first successful ventures in this endeavor employed sensitive Palmer levers that were arranged to trace a fine line on the smoked paper of a kymograph (Griffiths and Tauber 1943, Davey 1962, Brown 1965). In these preparations the active visceral organ was generally suspended by fine threads in a cylindrical muscle bath that received a stream of air. The lever was attached to one of the suspending threads. In recent years the measurement of movement has been achieved with a variety of electronic devices on the larger species of orthopterans (Cook and Holman 1978, 1979, Girardie and Lafon-Cazal 1972); lepidopterans (Cook et al. 1980, Platt and Reynolds 1986); and dipterans (Cook 1981, Cook and Meola 1978). However, most force transducers and strain gauges that have been developed since the 1950's lack the sensitivity to accurately detect forces in the high microgram and low milligram ranges. Indeed, the quantitative measurement of contractile force from the visceral organs of a small insect like the stable fly, *Stomoxys calcitrans* (L.), still presents a challenge. Although impedance converters have been successfully used to detect activity changes in the hindgut (Holman and Cook 1979) and heart (Cook and Meola 1983) of the stable fly, these changes were not calibrated in units of force.

This report describes a minature force transducer system that can both detect and quantitate the contractile events of the hindgut and oviduct of the stable fly. Representative myographs for each of these tissue types are presented.

## Materials and Methods

The measurement system was developed and tested in the laboratory, and the muscle preparations used in the study were obtained from adult female stable flies reared in a laboratory colony at the ARS-USDA, Veterinary Entomology Research

[1] Accepted for publication 31 August 1989.

[2] Texas A&M University, Central Machining and Electronic Repair Facilities, Mail Stop 3576, College Station, TX 77843.



http://prime-pdf-watermark.prime-prod.pubfactory.com/ | 2025-01-24

Unit, College Station, TX. In this colony both male and female flies were held together in screen cages at 27°C and 50% R.H. Flies were fed daily by placing cotton pads soaked with citrated bovine blood on top of the screen cages. At severn days after adult emergence, female flies needed for the tests were removed from cages in the colony.

The composition of saline used for dissection and perfusion was (in mM): sodium citrate 15, NaCl 75, $KNO_3$ 5, $CaCl_2$ 5, $MgSO_4.7H_2O$ 3, glucose 28, and histidine 10. The pH was adjusted to 6.8 with sodium hydroxide and the measured osmolarity was 238mO. Proctolin (Agr-Tyr-Leu-Pro-Thr) for the pharmacological test on the hindgut was obtained from Sigma Chemical Co., St. Louis, MO.

**Mechanical Transduction and Amplification.** The transducer used in the measurement system to detect the small muscle and/or organ movements was a Kulite BG10 miniature load cell (Kulite Semiconductor Products, Leonia, N.J.). This device is designed to measure a bi-directional tension force applied to the end of an ultraminiature cantilever beam. The beam is insensitive to lateral force inputs and its unique construction includes a Wheatstone bridge with 2 active arms that provide a combination of high sensitivity and high spring constant. The load cell (input impedance = 1800Ω) also contains mechanical stops to permit extreme overloading without damage.

The output signal of the load cell was enhanced to an appropriate level for detection and recording on a chart recorder with an electronic amplifier circuit (Fig. 1A). The following control features were incorporated into the amplifier design: (1) a gain select switch, (2) a zero adjust, and (3) a dampening control switch. The whole system inclusive of the load cell was powered by two 9 volt alkaline batteries through a pair of voltage regulators. the batteries lasted in excess of 40 hrs. When the amplifier was initially set up to register voltage changes produced by the strain guage, it was essential to set the output voltage at pin 1 to zero. This was accomplished by placing the strain guage in a vertical position and then adjusting Rl to give the appropriate zero reading at pin 1. Once adjusted Rl does not need to be changed again unless another load cell is used.

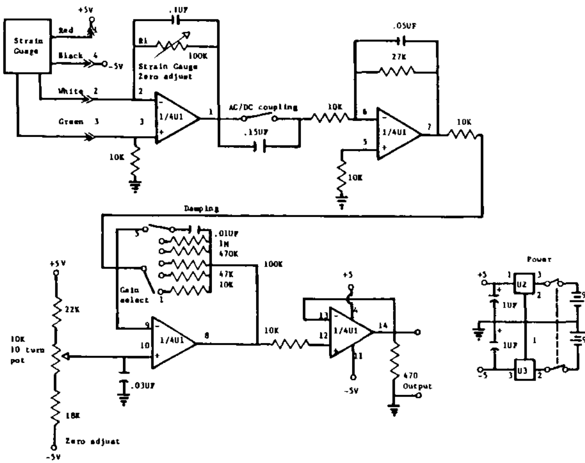
**Transducer Calibration.** The transducer recording system was calibrated by constructing a series of milligram weights (accurate to ± 50 μg) with aluminum foil. The load cell was then arranged in a horizontal position and the weights were placed over the hook attached to the cantilver beam. Five consecutive measurements were taken of the series of weights and a curve was constructed. A linear response was obtained for the range of forces tested (Fig. 1B).

**Preparation of Tissue and Myographic Recording.** Female flies were immobilized by chilling, placed in a small wax-filled Petri dish, and pinned through the ventral surface of the thorax. The minuten pin was thrust through the thorax close to the articulation of the wing to avoid striking the thoraci ganglion. The head, wings, and legs were removed and the central portion of the integument on the ventral abdomen was cut out. The body cavity was flooded with stable-fly saline and the integument of the admominal side walls was pinned with a minuten on each side.

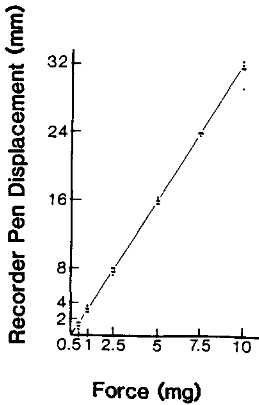
The hindgut was initally exposed by pulling the entire digestive tract in a posterior direction while carefully cutting tracheal attachments in the process. The ovaries and oviduct were removed by drawing them in a posterior direction and servering them at the level of the spermatheca. At this point the ovipositor was drawn out and pinned to the wax preparation dish with a minuten pin. This

http://prime-pdf-watermark.prime-prod.pubfactory.com/ | 2025-01-24

# A Amplifier Circuit Diagram




# B Calibration Curve for Transducer System




# C Experimental Arrangement

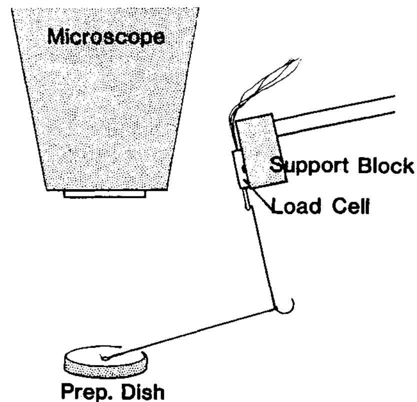



Fig. 1. Mechanical transducer - amplifier system. A) Schematic diagram of the amplifier shows a Quad operational amplifier (Ul) (type LM 324 or equivalent) and two voltage regulators (U2) a+5 volt regulator type 78L05 and (U3) a-5 volt regulator type 79L05. B) Calibration curve. C) Experimental arrangement for force measurements of hindgut and oviduct-preparations from the stable fly.

http://prime-pdf-watermark.prime-prod.pubfactory.com/ | 2025-01-24

procedure was followed by cutting the integument of the ovipositor along the midline to give more exposure to the terminal end of the hindgut. After the alimentary canal was cut off above the Malpighian tubules, the hindgut was ready for recording spontaneous muscle activity. The tissue was then connected to the force transducer by tying the intestine to a fine thread (a 22 μ monofilament of nylon, Ethicon Inc., Somerville, NJ) as shown in Figure 2A. The other end of the thread was looped over a steel hook attached to the beam of the load cell (Fig. 1C). In the final experimental arrangement it was essential to obtain an angle of 90° between this attached thread and the steel hook extending from the beam of the load cell.

When spontaneous muscular activity of the oviduct was to be recorded, the digestive tract was carefully removed by severing the hindgut from its close apposition to the oviduct near the spermatheca. The entire oviduct was then exposed by drawing it in a posterior direction while cutting the tracheal attachments. After the ovipositor was pinned in wax as described and the integument of that structure opened along the midline the oviduct was almost completely exposed as required for recording (Fig. 2B). In a manner similar to the hindgut preparation, a monofilament of nylon was tied about the midline of the ovaries and the other end of the filament was attached to the beam of the load cell (Fig. 1C). Once the hindgut and oviduct preparations were arranged in the 100 μl bath of saline as shown in Figure 1B, they remained active for as long as 6 h with only occasional changes of saline. Composition of the saline solution reported here was developed from a recent analysis of cations and anions found in adult stable fly hemolymph (Chen 1989).

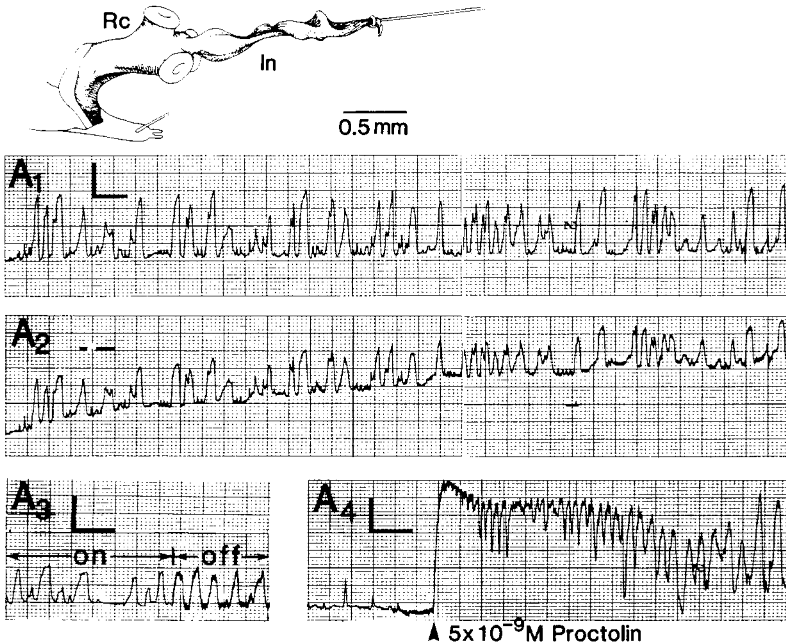
When it was desirable to compare impedance changes with force measurements simultaneously, two 36 guage silver wires leading to an impedance converter (UFI model 2991, Morro Bay, CA) were brought close to the suspended organ. Any small movement of the organ could then be registered as an impedance change and recorded graphically on a chart recorder.

## Results and Discussion

Movement is an essential property of the organic action of animals, and biologists have long acknowledged that the elongate muscle cell is the instrumental means for such locomotion. The remarkable power of these cells rests in their ability to shorten in response to the various stimuli. In insects muscles can be divided functionally into two broad classes: skeletal muscle and visceral muscle. Skeletal muscles move the appendages and are generally stimulated by nerves. Visceral muscles, by comparison, often contract spontaneously in the process of propelling food through the alimentary system or eggs through the oviduct. Nevertheless, the muscular activity of these tubular organs is also regulated by nerves (Cook and Holman 1979).

Detailed and precise knowledge on the mechanical activity of visceral muscles of insects will certainly contribute to our understanding of muscle physiology. However, obtaining such information from visceral organs of small insects has presented a formidable challenge until now. Impedance conversion devices have been useful in the detection of visceral muscle activity in such insects but the contractions could not be quantitated in units of force (Holman and Cook 1979). Nevertheless, a direct comparison of force transducer measurements with impedance

http://prime-pdf-watermark.prime-prod.pubfactory.com/ | 2025-01-24



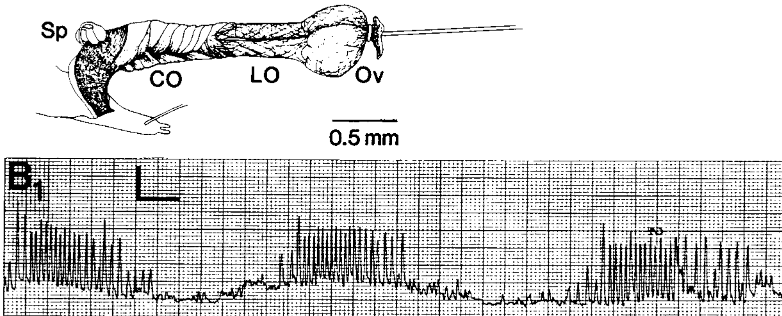




Fig. 2. Myographs obtained with transducer system. A) Suspension of stable fly hindgut for force measurement (RC = rectum, In = intestine). $A_1$) Record of spontaneous changes in hindgut contractile force. $A_2$) A simultaneous impedance myograph of hindgut activity for the same time shown in $A_1$. $A_3$) Effect of amplifier dampening function. $A_4$) Effect of proctolin on hindgut activity. B) Suspension of stable fly oviduct for force measurement (CO = common oviduct, LO = lateral oviduct, OV = ovary). $B_1$) Myograph of spontaneous changes in oviduct contractile force. The vertical calibration = 5 mg in $A_1$ and $A_3$ and 2.5 mg in $A_4$ and $B_1$. Each horizontal calibration = 10 sec.

http://prime-pdf-watermark.prime-prod.pubfactory.com/ | 2025-01-24

conversion data from the same stable fly hindgut preparation shows a remarkable similarity between the two contraction profiles (Fig. 2A, $A_1$ and $A_2$). The slight time lag between the spikes on $A_1$ and the spikes on $A_2$ is probably a consequence of differences in recorder pen alignments. The myograph shown in $A_1$ was obtained with the Kulite Load cell, which registered contractile force, while the myograph in $A_2$ shows a simultaneous record of impedance changes. thus in certain physiological and pharmacological studies where only a semiquantitative estimate of spontaneous activity or drug action is required the impedance converter would seem quite reliable.

A dampening function was incorporated into the amplifier circuit to reduce electronic noise (Fig. 1A). Its utility is illustrated in Figure $2A_3$. Initially, in this trace the dampening function was engaged as evidenced by the thin pin line but this line became broader once the dampening was turned off.

The contraction of visceral muscles of insects represents the end-point in a series of chemical and physical changes that occurs in cells either spontaneously or as a consequence of neural or hormonal stimulation. The recording of mechanical events associated with these contractions are valuable in establishing the existance of cellular receptors for chemical messengers. Proctolin (Agr-Tyr-Leu-Pro-Thr) a myotropic peptide of rather broad distribution among insects (Brown 1977) causes visceral muscles to contract. It has been reported in whole body extracts of the stable fly at a concentration of $12.4 \pm 0.66$ ng/gm of insect tissue, and it was found to cause a detectable increase in the frequency and amplitude of spontaneous contractions in the hindgut of the fly at a concentration of $5 \times 10^{-9}$ M (Holman and Cook 1979). However, we found a ten fold increase in response of the hindgut with the same concentration of peptide when the present recording system (Fig. $2A_4$) was used which suggests that the actual threshold for proctolin on the hindgut may be as low as $5 \times 10^{-10}$ M. These data suggest that the new transducer recording system as described can be used to generate more precise information in studying the pharmacological properties of endogenous chemicals that regulate visceral muscle.

Changes in the level of excitability within insect visceral muscle cells are reflected as spontaneous myogenic contractions. We have shown that in the longitudinal muscles of the stable fly hindgut (Fig. $2A_1$) there is usually an irregular pattern of slow monophasic contractions together with a smaller number of rapid phasic contractions of shorter amplitude. The base line tonus remained relatively unchanged. The contraction profile of the stable fly oviduct was quite regular compared to the hindgut. The duration of monphasic contractions was consistently shorter and occasionally appeared in trains (Fig. $2B_1$). There was also an evident flux in the base line tonus. this dichotomy in contraction patterns in the two tubular organs is interesting because of their structural similarity and close anatomical relationship.

In summary the system we have developed should prove quite useful for the measurement of the contractile force of small muscles and organs found in many insects.

**Acknowledgment**

We wish to thank Dr. Kenneth Beerwinkle and Dr. Hilary Goonewardene for their critical review of this manuscript.

http://prime-pdf-watermark.prime-prod.pubfactory.com/ | 2025-01-24

## References Cited

**Brown, B. E.** 1965. Pharmacologically active constituents of the cockroach corpus cardacum: Resolution and some characteristics. Gen. Comp. Endocr. 5: 387-401.

**Brown, B. E.** 1977. Occurrence of protolin in six orders of insects. J. Insect Physiol. 23: 861-864.

**Chen, A. C.** 1989. Changes in the hemolymph of the stable fly, *Stomoxys calcitrans,* after a blood meal. Arch. Insect Biochem. Physiol. 11: 147-158.

**Cook, B. J.** 1981. The action of proctolin and 5-hydroxytryptamine on the oviduct of the horse fly, *Tabanus proximus.* J. Invert. Reprod. 3: 209-212.

**Cook, B. J. and G. M. Holman.** 1978. Comparative pharmacological properties of muscle function in the foregut and the hindgut of the cockroach *Leucophaea maderae.* Comp. Biochem. Physiol. 61C: 291-295.

**Cook, B. J., and G. M. Holman.** 1979. The pharmacology of insect visceral muscle. Comp. Biochem. Physiol. 64C: 183-190.

**Cook, B. J., and S. Meola.** 1978. The oviduct musculature of the horsefly, *Tabanus sulcifrons*, and its response to 5-hydroxytryptamine and proctolin. Physiol. Entomol. 3: 273-280.

**Cook, B. J., Thompson, J. M., and W. D. Shelton.** 1980. Some structural and functional properties of nerves and muscles in the oviduct of the pink bollworm moth, *Pectinophora gossypiella* (Saund.). Int. J. Invertebrate Reprod. 2: 351-362.

**Cook, B. J., and S. Meola.** 1983. Heart structure and beat of the stable fly, *Stomoxys calcitrans.* Physiol. Entomol. 8: 139-149.

**Davey, K. G.** 1962. The mode of action of the corpus cardiacum on the hindgut in *Periplaneta americana.* J. Exp. Biol. 39: 319-324.

**Girardie, A., and M. Lafon-Cazal.** 1972. Controle endocrine des contractions de l' oviducte isole de *Locusta migratioria migratorioides.* C. R. Acad. Sci., Series D., 274: 2208-2210.

**Griffiths, J. G., and O. E. Tauber.** 1943. Effects of pH and of various concentrations of sodium, potassium, calcium chlorides in muscular activity of the isolated crop of *Periplaneta americana* (Orthoptera). J. Gen. Physiol. 26: 541-558.

**Holman, G. M., and B. J. Cook.** 1979. the analytical detemination of proctolin by HPLC and its pharmacological action in the stable fly. Comp. Biochem. Physiol. 62C: 231-235.

**Platt, N., and S. E. Reynolds.** 1986. the pharmacology of the heart of a caterpillar, the tobacco hornworm, *Manduca sexta.* J. Insect Physiol. 32: 221-30.

---

http://prime-pdf-watermark.prime-prod.pubfactory.com/ | 2025-01-24