perimysium. In addition, it may be expected that the area of overlap between the actin and myosin filaments within the myofibrils is decreased, leading to a widening of the H- and I-bands. But the tension generated within the myofibrillae and opposing the stretching force, is potentiated by the activity of the muscle spindles. COOPER and DANIEL[4] have shown that these are active at all lengths and tensions of muscle.

In phase 2 stretch, the actin and myosin filaments continue to separate, but the rate of increase in length is reduced although the applied tension continues to rise. It may well be that the fine helical collagenous fibres of the endomysium surrounding the muscle fibrils are stretched in phase 2 until they lie parallel to the myofibrillae. These collagenous fibres are not elastic and their resistance to a stretching force is greater than that of the elastic fibres in the perimysium. This may account for the reduced rate of lengthening which occurs in phase 2.

In phase 3 (stretch) the myofibrils are maximally stretched and no further elongation can occur without their disruption. The helical fibres of the endomysium are also fully extended and protect the myofibrils by their resistance to stretch. The result is that no further increase in the length of the muscle occurs although the applied tension may continue to rise.

When tension is relaxed, phases 2 and 3 are prolonged and the muscle is still longer at the end of phase 1 than it was at the beginning of the experiment. Probably a slow re-arrangement of muscle collagen occurs from a stretched parallel pattern to the unstretched helical form. This prevents the muscle from regaining its normal length immediately applied tension is relaxed. The inelastic collagen bundles require active muscle contraction in order to restore their arrangement to that present in the resting muscle before it was stretched.

A large part of the literature on the physiology of muscle is devoted to the maximum tension generated on nervous stimulation. In contrast, it is emphasized here that a muscle does not behave like an elastic band when it is stretched, a matter of considerable orthopaedic importance. In orthopaedic practice tension is applied to the limb as a whole when attempts are made to stretch muscles. Because the maximum possible increase in muscle length, at the most 20%, has been achieved by the end of phase 2, further increase in tension can have no effect on the muscles as far as increasing their length is concerned. Other structures in the limb such as the nerves and joints as well as the muscles themselves may be injured when subjected to forceful stretching. The tendency to increase the force applied to the limb in order to achieve greater lengthening of muscle should therefore be resisted. Not only can it but fail to lengthen muscle further; it may be positively harmful.

Zusammenfassung. Nachweis, dass sich der Skeletmuskel bei Streckung dreiphasisch verhält, wobei sich in der letzten Phase seine Länge auch bei grösserem Zug nicht mehr ändert.

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[4] S. COOPER and P. M. DANIEL, Brain *86*, 563 (1963).

Water Movement During Diuresis in the Tsetse Fly (*Glossina austeni*)

Tsetse flies take blood meals at least equal to their own weight at regular intervals[1]. This imposes severe limitations on the flying ability of the fly[2], and it is therefore advantageous that the fly should eliminate the water from the blood meal in the shortest possible time. In fact, *Glossina* often begins diuresis in the first minute following the commencement of feeding and it has been shown that in male *Glossina*, 38% of the total weight of the blood meal is excreted during the 30 min after feeding[3]. Diuresis in female *G. austeni* is even more rapid with more than 40% of the total meal weight being excreted in the first half hour after feeding (unpublished observations). The excretion of water is more rapid than that reported for other haematophagous insects and the speed with which the first drops of urine appear led me to investigate the problem of water movement during diuresis in female *G. austeni*. Did water from the blood meal in fact pass into the haemolymph to be coincidently removed by the Malpighian tubules, under the influence of diuretic hormone?

LESTER and LLOYD[4] were the first authors to describe the role of the Malpighian tubules in excretion in tsetses and based their results on observations of water movement within the tubules during diuresis. Prior to this, however, NEWSTEAD, DUTTON and TODD[5] had hypothesized that the rapid excretion of water in *Glossina* resulted from the clotting of the blood meal in the midgut with the subsequent passage of serum directly down the gut. LESTER and LLOYD[4] discounted this hypothesis by feeding flies on both haemolyzed blood and on blood containing methylene blue and by observing that neither the haemoglobin nor the dye appeared in the urine. However, their experimental results are not convincing because of the unphysiological conditions used for feeding and therefore, I have observed the excretion of both ^3H_2O and a large, presumably unmetabolized molecule, ^{14}C-dextran, following feeding.

Materials and methods. Female *G. austeni*, kindly supplied by Dr. P.A. LANGLEY, during their second reproductive cycle, were fed on fresh defibrinated bovine blood after the method of LANGLEY[6] and MEWS (unpublished), containing 125,000–145,000 dpm ^3H_2O (NEN, specific activity 1 μCi/μl) per μl and 1500 dpm ^{14}C-carboxyl dextran (NEN, specific activity 781 mg/mCi, M.W. 60,000–90,000) per μl. The flies had been previously reared on goats and were fed on the previous day, in one instance, and 2 days previous in another instance. Samples of urine were taken after 5 min, 15 min and 30 min from the cessation of feeding and placed directly into scintillation vials containing 10 ml of 1% butyl-PBD, 10% ethanol and 50 m*M* acetic acid in toluene. Samples were counted in a Packard Tricarb Liquid Scintillation Spectrometer using doublelabel settings and both quench correction and spillover correction were performed using external standardization. In some instances, samples of haemolymph were also obtained from

[1] S. S. TOBE and K. G. DAVEY, Can. J. Zool. *50*, 999 (1972).
[2] J. P. GLASGOW, J. Anim. Ecol. *30*, 77 (1961).
[3] S. K. MOLOO and S. B. KUTUZA, Acta trop. *27*, 356 (1970).
[4] H. M. O. LESTER and L. LLOYD, Bull. ent. Res. *19*, 39 (1928).
[5] R. NEWSTEAD, *Guide to the Study of Tsetse-Flies* (Liverpool School Hyg. trop. Med. 1924), Memoir No. 1.
[6] P. A. LANGLEY, Bull. ent. Res. *62*, 215 (1972).

Radioactivity in dpm/μl in urine and haemolymph of *G. austeni* after feeding on blood containing 125,000–145,000 dpm ^3H_2O per μl and 1500 dpm ^{14}C-dextran per μl

	Time post-feed (min) 5	15	30
Urine			
^3H	122,620 ± 24,197 (31)	119,893 ± 26,335 (13)	117,304 ± 50,313 (4)
^{14}C	0 - 1500 (31) [a]	0 (13)	0 (4)
Haemolymph			
^3H	75,490 ± 16,726 (10)	81,700 ± 7,426 (10)	—
^{14}C	0 (10)	0 (10)	—

[a] 13 out of 31 flies excreted ^{14}C. The range of the specific activity in the urine was between 0 and 1500 dpm/μl., with 3 flies excreting urine with the same specific activity as the blood meal and the remaining 10 flies excreting urine with intermediate values. ± standard deviation. Figures in brackets represent *n*. No protein was observed in any urine samples

flies 5, 15 and 30 min after feeding by cutting off the wing and gently squeezing the fly. These samples were also counted by the above scintillation techniques. Protein in the urine was determined on 2 μl aliquots from 20 flies at 5, 15 and 30 min after feeding by the Xylene brilliant cyanin G method of BRAMHALL et al[7]. The results of these experiments are presented in the Table.

Results and discussion. It is apparent form the Table that the specific activity of the urine 5 min after feeding approximates that of the blood meal while that in the haemolymph is significantly less ($p < 0.001$). This difference in specific activity of urine and haemolymph can also be observed at 15 min ($p < 0.025$). It is also striking that some ^{14}C counts are present in the urine of at least some flies, albeit in varying quantities. It is not known if decarboxylation of the ^{14}C-dextran occurs in the gut, but because no ^{14}C appears in the haemolymph, there is no reason to believe that this is the case. No protein was detectable in any samples of urine and these results are consistent with the findings of LESTER and LIOYD[4].

The fact that the specific activity of the urine, in the case of ^3H, is not significantly different from that of the blood meal, suggests that there exists in tsetse flies, some undescribed and relatively direct method for the rapid removal of excess water from the blood meal. If the conventional method of diuresis described in *Rhodnius*[8] were operating in *Glossina*, with water passing through the gut wall into the haemolymph and being subsequently removed by the Malpighian tubules, one would predict that the specific activity of the haemolymph would be at least equal to that of the urine. Taking into consideration the exchange of labelled and unlabelled water molecules in the cells of the Malpighian tubules and the hindgut, one would further predict that the specific activity of the urine would be less than that of the haemolymph. This is not the case.

I suggest that there exists in the body of *Glossina*, a compartmentalization of water during the period of diuresis which prevents the massive flow of water through the entire haemolymph. It is possible to construct a model of the system where one possible route of diuresis involves the passage of water directly down the gut, with water moving within the gut lumen (possibly coincident with the 'clotting' of the blood meal and subsequent expression of water from the meal or through the use of the peritrophic membrane as a semi-permeable membrane). This model is supported by the fact that at least some flies excreted ^{14}C-dextran at concentrations approaching that in the blood meal. Alternatively, the system may involve the existence of compartments within the haemolymph (possibly through the intimate association of midgut with Malpighian tubules or through the use of a barrier to physical diffusion), such that a closed loop condition is realized during diuresis, with discrete pools of the haemolymph (i.e. compartmentalized portions) in only local equilibrium with water in the tissues and not with the entire haemolymph. This model is supported by the suggestion that the abdominal organs of *G. austeni* are covered by only a thin film of haemolymph[1]. Although it is not possible to choose between the two models at present, it is clear that a barrier(s) exists during diuresis in *G. austeni* which prevents the free exchange of water from the blood meal with that in the haemolymph, and preliminary observations on *G. morsitans* indicate a similar phenomenon may occur in this species.

Thus, the general composition of the haemolymph after feeding may undergo little change because the flow of water through the entire haemolymph has been impeded through the use of compartments within the animal. Although the mechanism of this process remains unelucidated, such a phenomenon may also occur in other haematophagous insects and if so, the whole process of diuresis in insects may require re-evaluation.

Résumé. Quand les mouches tsétsé (*Glossina austeni*) se nourrissent de sang contenant du ^3H_2O et du ^{14}C-dextran, le ^3H_2O réapparaît après 5 min dans l'urine à des concentrations qui se rapprochent de celles de son absorption. La concentration du ^3H_2O dans l'hémolymphe est nettement moins élevée que dans l'urine. Le ^{14}C-dextran est présent au moins dans l'urine des mouches. Ces résultats suggèrent un compartimentage de l'eau durant la diurèse, ce qui prévient la dilution de l'hémolymph.

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[7] S. BRAMHALL, N. NOACK, M. WU and J. R. LOEWENBERG, Analyt. Biochem. *31*, 146 (1969).

[8] S. H. P. MADDRELL, Adv. Insect Physiol. *8*, 199 (1971).

[9] I thank Dr. P. A. LANGLEY for valuable discussion and for assistance with experiments at the Tsetse Research Laboratory, University of Bristol. I also thank Prof. K. G. DAVEY, Dr. S. H. P. MADDRELL and Dr. G. E. PRATT for discussions and comments on the manuscript. This work was initiated at the Institute of Parasitology, McGill University. Receipt of a National Research Council of Canada post-doctoral fellowship is acknowledged.