hornworms were reared on semi-synthetic diet and excreta of the 5th instar larvae was used in this investigation. One dimensional ascending and descending chromatography was used to separate the nitrogenous compounds. Different solvent system used were: Isopropynol-water (10:3), ethanol-acetic acid-water (85:5:10), butanol-methanol-benzene-water (2:1:1:1) and ethanol-pyridine-water (70:20:10). Whatman No. 1 paper was used for all chromatograms.

The samples for urea and allantoin were prepared by grinding the faecal pellets in a mortar with 0.067 *M* phosphate buffer pH 12[5] and with 0.4% lithium carbonate for purines and pyrimidines[2]. The resulting suspensions were then centrifuged; supernatant was concentrated and aliquots from it were taken for chromatographic analysis. Chromatograms were air dried and sprayed with the mercury-diphenyl-carbazone reagent of DIKSTEIN et al.[8] to reveal purines and pyrimidines. These compounds could also be detected by viewing the chromatograms under UV- light. Urea and allantoin were detected by spraying the chromatograms with dimethylamine benzaldehyde reagent[9]. Identification was made by comparision of Rf values of known compounds.

On the basis of the colour development it can be inferred that urea was present only in traces; uric acid and allantoin being the main constituents of nitrogenous waste products of *Manduca*.

Since insects in general are uricotylic, it is not surprizing to find that uric acid is one of the excretory products in Tobacco hornworm. Allantoin which is present in the hornworm excreta has also been reported in other lepidopterous insects[10]. It seems that the enzyme uricase which is responsible for the breakdown of uric acid to allantoin[10,11], is also operating in this insect. Urea which was found in traces in hornworm excreta is reported to be a minor excretory product in many insect[2].

Zusammenfassung. Die stickstoffhaltigen Exkretionsprodukte von Raupen von *Manduca sexta* L. wurden papierchromatographisch analysiert. Die wichtigsten Ausscheidungsprodukte sind Harnsäure und Allantoin; Harnstoff wird nur in Spuren ausgeschieden.

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Ureteral Pacemaker Potentials Recorded with the Sucrose Gap Technique

Presence of the renal pelvis in isolated ureteral preparations is essential for the continuation of regular peristaltic waves in vitro[1,2]. CONSTANTINOU[3] has reported rhythmic contractions in the dog renal pelvis, and together with GOLENHOFEN and HANNAPPEL[2] has found multimodal distributions of the period between peristaltic waves. Thus, a pacemaker region may well be located in the renal pelvis, where morphological differentiation has also been found[4]. The aim of the present work was to record pacemaker potentials in the renal pelvis electrically, and to relate them to the contractile behaviour of the ureter in vitro.

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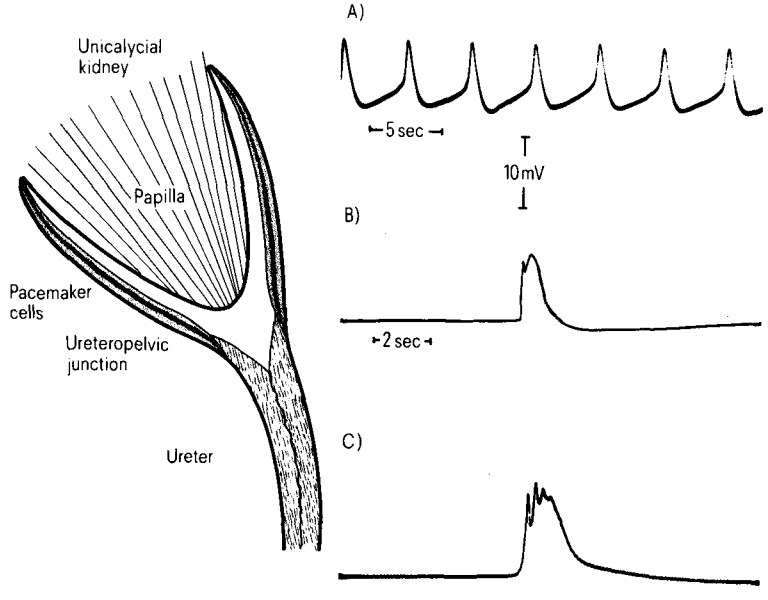

Fig. 1. Sucrose gap records of electrical activity from the regions of the guinea-pig ureter shown in the diagram. A) Pacemaker potentials from the renal pelvis. B) Transitional action potential from the ureteropelvic region. C) Propagated action potential from middle ureter.

Materials and methods. Guinea-pig ureters were dissected with renal pelvis intact, and set up immediately in a single sucrose gap apparatus, as described by BURNSTOCK and STRAUB[5] with tension simultaneously monitored by a Statham transducer. Sheep upper ureters were brought from the abbatoirs in cold Krebs solution and the preparations were set up within 1h of the death of the animal. Some experiments were carried out in an organ bath, and the tension changes were recorded on a Nihon Kohden chart recorder.

Results and discussion. The membrane potential fluctuations detected in the various anatomical sites of the proximal ureter with the sucrose gap method are shown in Figure 1. The potentials recorded at the renal pelvis (Figure 1 A) are similar to those found in the SA node of the heart, beginning with a slow prepotential whose rate of rise increases, ultimately leading into a spike. The pacemaker frequency in both the guinea-pig and sheep was of the order of 0.22 Hz. The records in Figures 1 B and 1 C were obtained from the pyeloureter (ureteropelvic junction) and the middle ureter respectively. As the recording site was shifted away from the renal pelvis, the pacemaker potentials disappeared and the action potentials were characterized, instead, by a fast rate of rise. In the guinea-pig ureter, spikes were generally superimposed on a plateau. The non-pacemaker types of action potentials recorded here were similar to those obtained by GOLENHOFEN and HANNAPPEL[6] with microelectrodes, and by BURNSTOCK and PROSSER[7] with the sucrose gap. Pacemaker depolarizations in the renal pelvis, however, have not been previously demonstrated.

Microelectrode measurements from the guinea-pig renal pelvis are difficult since that region spontaneously contracts, and at a much faster rate than that of the peristaltic waves. Furthermore, due to the very low tension developed by the pacemaker area of the guinea-pig ureter, the relation between the contractions in the renal pelvis and the electrical activity of the pacemaker was studied in the sheep proximal ureter. The upper, tension, record in Figure 2 initially bears a 1:1 relationship with the electrical pacemaker potentials, but then dissociates into two smaller components which show an increasing phase difference until synchrony reoccurs. This result suggests the existence, in that preparation, of two unsynchronized areas of renal pelvis contracting at slightly different frequencies. The pacemaker potentials occurred at a constant frequency, since the sucrose gap recording method does not distinguish small areas of poorly coupled independent activity. Furthermore, the relative contribution of the various potential pacemaker regions to the total pacemaker process may be determined by the degree of electrical coupling between these areas[8], and may be grossly influenced by the dissecting procedure. In this context, CONSTANTINOU[3] has confirmed that, in dog renal pelvis, pressure in vivo fluctuated with no asynchronous component.

The relation between pacemaker potentials and ureteral peristaltic waves is shown in Figure 3, which indicates that not every pacemaker potential triggers a peristaltic wave in the ureter. The correlation between pacemaker potentials and peristaltic waves was studied by plotting a frequency histogram of intervals between successive contractions of the ureter after the manner of GOLENHOFEN and HANNAPPEL[2,8]. The usual multimodal distribution was obtained, as in Figure 4 A. However, the minimum interval between contractions was shorter than previously suggested values[2,8], being near 4.55 sec. Integral multiples of this value then gave peaks at 9.1, 18.2 and 27.3 sec, differing by −9%, 4% and 9% from those found in situ by GOLENHOFEN and HANNAPPEL[2,8], so that the deviations of up to 25% from integral multiplication of the pacemaker period in vivo reported by these authors, might be explained by a pacemaker fre-

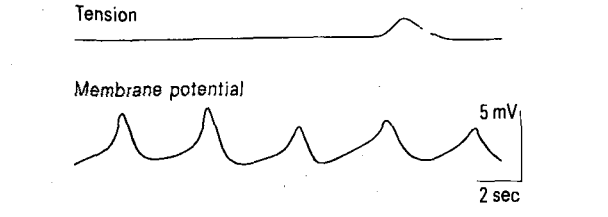

Fig. 3. Relation between pacemaker potentials (lower trace) and tension (upper trace) resulting from peristaltic waves in the ureter. The record has been numerically rescaled to a slower timebase. Note the absence of a 1:1 correspondence between the two traces.

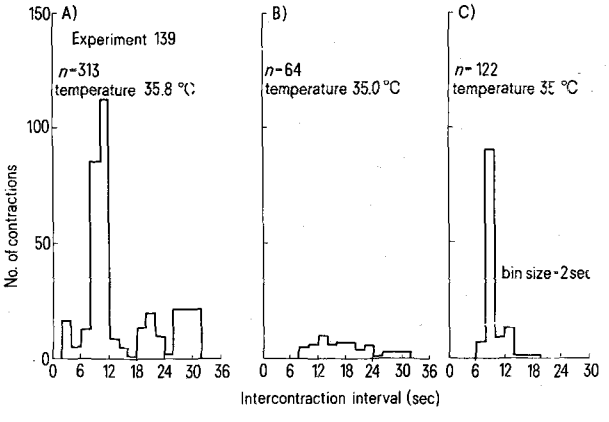

Fig. 4. Multimodal distributions of contraction intervals of guinea-pig ureter in vitro. A) Typical isolated ureter. Experiment duration 2 h. B) Different preparation from A) 30 min control run. C) Same preparation as in B), stretched by 25%. Recording time 30 min.

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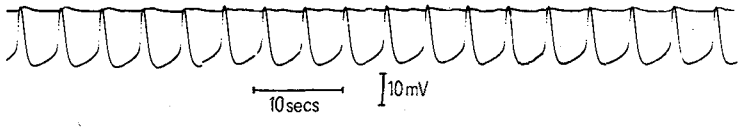

Fig. 2. Sucrose gap record of pacemaker activity in the renal pelvis of the sheep (lower trace) with concomitant tension record (upper trace). Note the phase difference between the two traces.

quency higher than the highest peristaltic rate recorded, instead of the reflex inhibitory mechanism which they proposed. However, the ureter has been examined from a feedback control systems point of view[9] and the existence of such a mechanism cannot be ruled out.

The pacemaker frequency in both guinea-pig and sheep was remarkably stable. The standard deviations of groups of 35 consecutive pacemaker potentials in the sheep ranged from 2% to 6% of the mean, so that the spread of the histograms is more likely to be due to variability of the threshold of excitation in the pyeloureter, which acts as a minor delay mechanism. The pyeloureter also acts as a gate, preventing most pacemaker potentials from initiating peristaltic waves.

Two possible influences on this proposed gating or filtering mechanism were investigated. Figures 4B and 4C show distributions obtained before and after 25% stretch was applied to the whole preparation. This allowed more contractions to occur at lower multiples of the pacemaker period, presumably by lowering the threshold of excitation in the pyeloureter. The action of different temperatures on the isolated guinea-pig ureter likewise changed the gate threshold. In the temperature range 29–33 °C, the distributions were unimodal with periods of 30 sec, but in the range 34–37 °C the distributions became multimodal, with peaks at approximately 30, 20 and 15 sec.

Thus, although the pacemaker is a necessary condition for the initiation of peristaltic waves, an additional stimulus in the pyreloureter, by way of distention due to accumulated urine in the renal pelvis, is required before the peristaltic wave can be triggered. Such a mechanism would effectively isolate the renal compartment hydrodynamically from the urinary bladder. During diuresis, however, the distension in the renal pelvis would lower the pyeloureteral gate excitation threshold in a manner similar to that demonstrated experimentally by the application of stretch as in Figure 4C. A full renal pelvis would therefore give rise to a unimodal distribution of peristaltic intervals, with a pacemaker to ureteral contraction ratio of 1:1. Under such conditions, hydrodynamic isolation of the kidney would still occur due to the continued maintenance of regular peristaltic contractions by the renal pacemaker.[10]

Summary. Ureteral contractions occur at intervals which are integral multiples of the period of pacemaker potentials recorded in vitro from the renal pelvis with a sucrose gap, suggesting that a gating mechanism in the pyeloureter regulates the rate at which the pacemaker initiates contractions.

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Inhibition of Light-Induced, Transient Membrane Potential Oscillations of *Oenothera* Leaf Cells by Cycloheximide

The antibiotic cycloheximide (CH), known as a specific inhibitor of protein synthesis in 80 S (cytoplasmic) ribosomes of eucaryotic cells[1], has recently been used in studies of ion uptake and transport in plants[2–5]. Besides protein synthesis, CH inhibits transport of ions through barley roots, whereas ion accumulation from the ambient medium is not affected[3,4]. It was suggested that CH exerts its effect on symplasmic ion transport in the root parenchyma by interaction with the endoplasmic reticulum (ER) and its membrane turnover[3,4]. In the experiments described here, we attempted to investigate whether CH also affects other symplasmic phenomena such as electrical coupling between neighbouring cells in plant tissues.

For demonstration of electrical coupling, we used the internally generated signal of the well-known transients of electrical potential difference (PD) which are triggered by switching on or off photosynthetic energy transfer reactions (review[6]). These PD transients also occur in normally green cells of variegated leaves of *Oenothera*-mutants. PD transients are not observed, however, in the yellowish mutated cells having an impaired photosystem I or photosystem II respectively, unless there is a symplasmic connection between the green and the mutated cells[7]. This shows that the light-triggered signal can be translocated from the green cells to the mutated cells.

In our experiments, we used micro-capillary electrodes (tip diameter < 1 µm, filled with 3 *M* KCl, resistance > 4 Mohms in 3 *M* KCl). The electrodes were inserted into the center (probably the vacuole) of a cell of the upper palisade parenchyma layer, after the epidermis was stripped off. The leaf sample was mounted into a small chamber which was continuously flushed with artificial pond water (APW: 1 m*M* NaCl + 0.1 m*M* KCl + 0.05 m*M* $CaSO_4$, pH about 6.1). For more experimental details see[8].

Initial experiments showed that after addition of CH (10 µg/ml = 36 µ*M*) to the external medium, no light-induced potential changes were detectable with the electrode tip in white cells of *Oenothera hookeri · albicans* IV/IIα, although green cells were in symplasmic contact with the mutated cells. Thus, apparently CH blocked symplasmic transfer of the signal.

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