IAA (2 mg/l) was added when similar extracts from cultures of different ages or the chromatographic bands were tested. After 4 weeks incubation at 24 °C, the fresh weight of callus in each treatment was determined. The effect of the preparations on the retention of chlorophyll by oat leaves cv. Criolla was tested using the method of THIMANN and SACHS[9]. Five 15 µl droplets (equivalent to 15 ml of culture filtrate) were used for each preparation.

If either IAA or kinetin was omitted from the control medium, there was little growth and the tissues soon turned brown. However, the crude ethyl acetate extract supported good growth in the absence of added plant hormones. Roots but not buds commonly formed, suggesting that the concentration of auxins and their ratio to cytokinin-like substances in the extract was high. The best growth was obtained with the 10-day culture filtrate (Table I). At this time, viability counts in the culture had begun to decline, after rising exponentially for 5 days and remaining in the stationary phase for another 4 days. Over the 12-day incubation period, the pH of the medium slowly rose from 6.6 to 7.8. Several of the chromatographic bands with Rf similar to known cytokinins, i.e., 6-(γ,γ-dimethylallylamino) purine and its ribonucleoside, also had cytokinin-like activity (Table II). In the oat leaf chlorophyll retention bioassay, the same bands were the most active.

Table II. Growth promotion of tobacco callus by individual bands from thin-layer chromatography of an extract from a 7-day culture of *P. savastanoi*

Band	Fresh wt. (g) [a]	Rf
1	0.88	0
2	0.44	0.05
3	0.65	0.09
4	1.08	0.17
5	2.86	0.29
6	0.83	0.38
7	0.99	0.41
8	0.52	0.55
9	0.79	0.63
10	0.56	0.74
11	0.51	0.80

[a] Average of 9 callus pieces. Culture filtrate equivalent to 12 l per l medium.

Table III. Growth promotion of olive callus by an ethyl acetate extract from a 7-day culture of *P. savastanoi*

Treatment	Fresh wt. (g) [a]
complete medium	0.70
IAA + kinetin + extract	0.98
NAA + extract	0.82
IAA + extract	1.28
kinetin + extract	1.01
extract	0.55

[a] Average of 9 callus pieces. Culture filtrate equivalent of 10 l per l medium.

The effect of the crude ethyl acetate extract on the growth of olive (cv. Cima di Mola) callus was also tested. Wood tissue from 1-year-old branches was grown in modified RM 1964 containing: (mg/l) IAA 2; α-naphthalene acetic acid (NAA) 1; kinetin 1; and ascorbic acid 1. On media lacking auxin or containing < 0.5 mg/l kinetin the callus soon turned brown and died. But, as with tobacco callus, the ethyl acetate extract supported good growth of olive callus in the absence of kinetin (Table III).

These results show that some substance(s) with cytokinin-like activity is synthesized by *P. savastanoi*. Perhaps it is also produced in developing galls where it might act in concert with auxins. However, the presence in galls of elevated levels of plant hormones, and their source, remain to be shown. Further work to elucidate these points and to identify the substance(s) in the extract having cytokinin-like activity is in progress.

Riassunto. Filtrati colturali parzialmente purificati del batterio *Pseudomonas savastanoi* hanno mostrato attività di tipo citocininico quando sono stati esaminati mediante sistemi di saggio biologico consistenti nella risposta di calli di tabacco e di olivo e nella ritenzione della clorofilla da parte di foglie senescenti di avena.

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Nitrogenous Excretory Products of Tobacco Hornworm, *Manduca sexta* (L.)

It has been shown by many workers that ammonia is the main nitrogenous excretory product in aquatic and semiaquatic insects[1], while in case of terrestial insects uric acid has been reported to be the main excretory product[2]. Purines other than uric acid are rarely found in insect excreta. Hypoxanthine and xanthine present in the excreta of *Melophagus ovinus*[3], *Drosophila melanogaster*[4] and *Galleria mellonella*[5,6] seem to reflect pecularities of purine metabolism. However, in case of cotton stainer, *Dystercus fasciatus*, the main nitrogenous excretory product was not uric acid but allantoin[7]. Urea, also has been found as a minor nitrogenous product in many insects[2].

Tobacco hornworm, *Manduca sexta*, was chosen for studying the various nitrogenous waste products because of its large size due to which sufficient quantities of excreta could be collected at short time intervals. The

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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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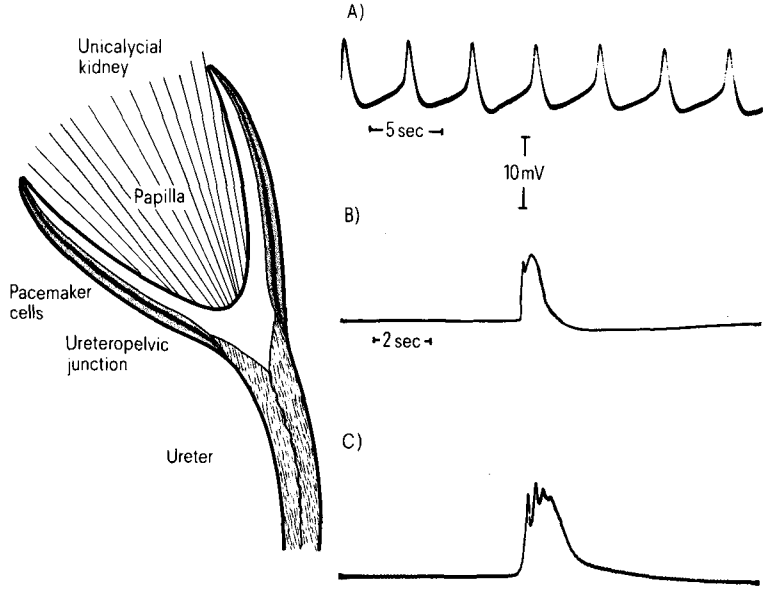

Fig. 1. Sucrose gap records of electrical activity from the regions of the guinea-pig ureter shown in the diagram. A) Pacemaker potentials from the renal pelvis. B) Transitional action potential from the ureteropelvic region. C) Propagated action potential from middle ureter.