tion. Saliva samples were collected by disposable micropipettes for a timed interval. For the determination of salivary calcium, 10 µl of saliva were used and titrated with ethylene glycol-bis(aminoethyl)-tetraacetic acid (EGTA) with the use of an automatic calcium titrator (Precision Systems). For the analysis of glandular calcium, immediately after 20 min of continuous stimulation of parasympathetic innervation, the stimulated parotid gland was removed rapidly and placed in a crucible and dry-ashed (550 °C for 18 h). The ash was dissolved in 0.5 ml of 1 N HCl and thoroughly mixed. This solution was used for determination of calcium concentration by the automatic calcium titrator. The unstimulated parotid gland of the contralateral side was used as a control and was treated in exactly the same way as the experimental gland.

Results. Data in figure 1 show that there were significant decreases in flow rate of saliva evoked by electrical stimulation of the parasympathetic innervation to the parotid gland of rats treated with amitriptyline (10 mg/kg i.p.) daily for 4 weeks as compared to those of untreated rats (fig. 1). However, parotids of rats treated with amitriptyline for 2 weeks produced a salivary flow in response to such nerve stimulation that was similar to that from glands of untreated rats (fig. 1). Chronic treatment with amitriptyline for either 2 weeks or 4 weeks had no effect on calcium concentration of saliva evoked by parasympathetic nerve stimulation (fig. 2). Chronic amitriptyline treatment did not cause any change in glandular calcium concentration of either control or stimulated parotid (table).

Discussion. The present study shows that chronic treatment with amitriptyline for 4 weeks significantly decreased salivary flow of parotid gland in response to electrical stimulation of the parasympathetic innervation without altering calcium concentration of nerve-evoked saliva. It has been established that there is a parallel decrease in flow rate and calcium concentration of saliva when intensity of stimulation to the parasympathetic innervation to parotid gland is decreased[6]. This presumably is the result of reduction in acetylcholine release. The dissociation between effects on flow rate and calcium concentration suggests that chronic amitriptyline administration may not necessarily be acting by this mechanism. An alternative explanation of these effects is that amitriptyline binds at the same membrane receptors normally occupied by acetylcholine.

Effect of chronic administration of amitriptyline on calcium concentration of rat parotid gland after parasympathetic nerve stimulation

Kind of stimulation	Glandular [Ca] (mEq/kg wet wt) Untreated	Chronic amitriptyline
None	11.80± 0.8 (5)	11.95± 0.2 (4)
Parasympathetic nerve	12.30± 1.0 (5)	11.60± 0.4 (4)

Values are means ± SE. Numbers in parentheses is number of experiments. Parasympathetic innervation to rat parotid (auriculotemporal nerve) was stimulated for 20 min.

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Circadian rhythms of serotonin and the electrical activity of the frontal ganglion of the cockroach, *Periplaneta americana*

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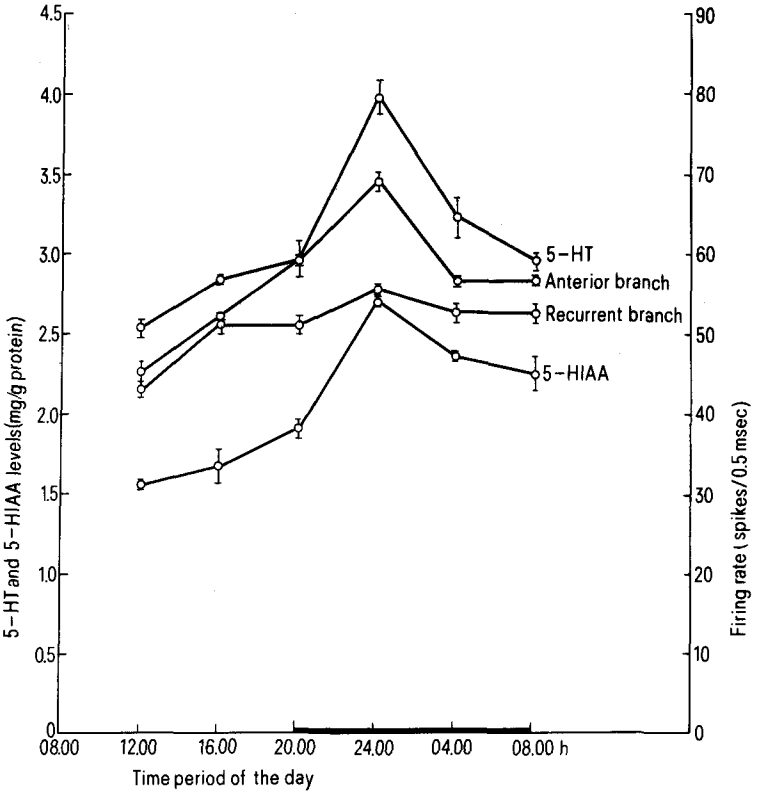
Summary. Rhythmic circadian variations in the spontaneous electrical activity of the frontal ganglion (FG) of the cockroach, *Periplaneta americana*, have been shown, and the neurotransmitter (NT) involved in this activity has been identified as serotonin (5-hydroxytryptamine, 5-HT). During the 24-h day, the diurnal variations in the electrical activity and the levels of 5-HT and its immediate metabolite 5-hydroxyindole acetic acid (5-HIAA) were maximal at 24.00 h and minimal at 12.00 h.

Circadian rhythms in activity are known to play a significant role in insect life. In the cockroach, diurnal rhythms in locomotor activity[1,2] and in spontaneous electrical activity and, in the acetylcholine content in nerve cord[3] have been reported. The FG is a part of the stomatogastric (SG) nervous system of the cockroach and has direct connections with the brain through 2 anterior branches and with the neuroendocrine (NE) system through a posterior recurrent branch. Since it is known that the FG is an autoactive tissue and it fires spontaneously without any signal input[4], its significance for the insect has yet to be established.

Materials and methods. Adult male cockroaches were used for the experiments. They were housed in a cage maintained at a temperature of 25 ± 1 °C in the laboratory. Food and water were supplied regularly. The insects were acclimated to laboratory conditions of 12 h light (08.00–20.00 h) and 12 h dark (20.00–08.00 h) for about 10 days before use in the experiments. The FG was dissected out and kept immersed in cockroach Ringer's solution[5]. Soft sodium glass capillary suction electrodes were used for activity recording whereby the electrical activity of the 2 anterior branches (diameter 100 µm) and that of the posterior recurrent branch (diameter 70 µm) was recorded separately using electrodes of similar diameter. The electrodes were filled with cockroach Ringer's solution and the anterior or the recurrent branch was sucked into the electrode tightly for the recording. To cover the 24-h period, 6 different times (04.00 h, 08.00 h, 12.00 h, 16.00 h, 20.00 h and



Circadian variations in the firing activity, 5-HT and 5-HIAA levels of the FG. The values of 5-HT and 5-HIAA are expressed as mg/g protein. The firing rate (FR) is expressed as the total number of spikes per 0.5 msec. Each value is the mean ± SE of 3 determinations. White and black bars at the baseline: light and dark periods of the day respectively. Analysis of variance: FR anterior branch-significant ($p < 0.01$); FR recurrent branch-significant ($p < 0.01$); 5-HT levels-significant ($p < 0.01$); 5-HIAA levels-significant ($p < 0.01$). Least squares difference method: FR anterior branch - r significant at 0.01 level (86% explained variations); FR recurrent branch - r not significant (55% explained variations); 5-HT levels - r significant at 0.01 level (90% explained variations); 5-HIAA levels - r significant at 0.05 level (81% explained variations).

24.00 h) were chosen to study the diurnal rhythm of activity. The signals were fed through a Grass model P15AC Preamplifier and displayed on Tektronix 5113 dual beam storage oscilloscope.

An improved fluorometric method[6] was adapted for the determination of 5-HT and 5-HIAA in the FG[7]. The protein content of the ganglion was estimated using bovine serum albumin as a standard[8] at all time-points.

Results and discussion. The levels of both 5-HT and 5-HIAA shown circadian variations. The minimum quantities of 5-HT and 5-HIAA were found at 12.00 h, and the maximum at 24.00 h (fig.). There was a rise in the levels from 12.00 to 24.00 h, and a gradual fall was observed from 24.00 to 12.00 h. At 04.00 h the levels of 5-HT and 5-HIAA were found to be significantly higher than their respective levels at 16.00 h.

The spontaneous electrical activity recorded from the anterior and the recurrent branches also shows variations throughout the day. The minimum firing activity (recorded in terms of firing frequency, i.e. number of total spikes in a particular time) was observed at 12.00 h, the maximum firing activity at 24.00 h (fig.). The firing pattern was the same in the anterior and the recurrent branch but the firing frequency was lower in the recurrent branch than that recorded from the anterior branch.

Since the FG is an autoactive tissue, the involvement of some NT in the autoactivity is suspected. Biogenic monoamines are known to occur in the SG nervous system of the insects[9-13]. The presence of 5-HT in the FG of the cockroach is in agreement with the identification of 5-HT in the nervous system of the cockroach by other investigators[14,15]. Serotonin has been recognized as a neurohormone in the insect nervous system[16,17]. Moreover, the levels of brain 5-HT have been found to be closely related with the characteristic behavioral changes, one of which is activity syndrome, and the increase and the decrease in the brain 5-HT levels in the cockroach lead to hyperactivity and hypoactivity, respectively[7,18]. Serotonin has been known to be involved in the rhythm regulation of the heart muscle cells of insects[19]. Circadian variations in the levels of 5-HT and 5-HIAA were closely associated with the rhythmic changes observed in the spontaneous electrical activity of the FG. Circadian changes in the brain 5-HT level have already been reported in certain insects[20,21]; this shows that the process of 5-HT synthesis takes place with the circadian rhythm.

It has been suggested that in the cockroach the locomotory activity rhythm may arise from the rhythms in the electrical activity of the CNS, and the neurohumors are thought to be the regulatory factors in the activity rhythms[22]. The rhythmic circadian variations in the electrical activity of the FG suggest that this firing activity is also largely influenced by the light-dark cycle of the day as is the case with 5-HT and 5-HIAA levels. Hence, the variations in the NT (5-HT) content may be one of the factors responsible for the observed variations in the firing activity. Rhythmic oscillations result from the complex interactions between nervous system, hormones and a variety of other factors[23]. The presence of inhibitory and the excitatory principles in the brain and corpora cardiaca of *Periplaneta americana* has been reported[24], and these principles could be of the nature of the neurohormones such as 5-HT. Thus, the synthesis and the release of 5-HT in the FG along with other possible neurohormonal factors may occur in a constant diurnal cycle, accounting for the diurnal rhythmicity in the electrical activity of this ganglion.

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Force development in smooth muscle strips of the hypertrophied urinary bladder of the rat after autonomic decentralization[1]

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Summary. Active length-tension relations for muscle strips of decentralized bladders differed from those of controls when comparisons were based on length in situ, but not when comparisons were based on lengths relative to optimum length for force development. The decentralized bladder behaved similarly to the denervated bladder, thus indicating that the presence of nerves was of no importance for the force production of directly stimulated muscle cells in the hypertrophied bladder.

A rat in which the urinary bladder is either denervated or decentralized is unable to pass its urine. During the first few postoperative days, when the bladder is manually emptied, the bladder gains markedly in weight[2,3]. Recently, it was shown that length-tension relations for muscle strips of denervated bladders, studied in an organ bath, differed from those of strips of control bladders[4].

In contrast to the denervated bladder, the decentralized bladder is still supplied with postganglionic nerves. In the present study we investigated whether the presence of these nerves is of importance for the ability of muscle strips from hypertrophied bladders to develop force, using AC current as the mode of stimulation.

Materials and methods. Ten male adult rats of a Sprague-Dawley strain were used; the operation was carried out on five of them. Under ether anesthesia and with the aid of a dissecting microscope, the preganglionic nerves of both the pelvic and the hypogastric ganglion were bilaterally cut[3]. The decentralized bladders were emptied manually once a day.

The animals were killed by cervical fracture, the operated ones 10–14 days postoperatively. The bladders were removed and transferred to oxygenated Krebs solution, with which they were also filled to a volume of 0.75 ml. A longitudinal section was marked out, measured and dissected out. The strips were mounted in the organ bath of an apparatus in which muscle force and shortening could be measured[5]. Force and length output were recorded on a Devices MX 4 linear direct-writing oscillograph. The bathing medium was a Krebs solution of the following composition in mM: NaCl 115, KCl 4.73, $CaCl_2$ 2.5, $NaHCO_3$ 15.5, KH_2PO_4 1.19, and glucose 11.5. It was kept at 37 °C and bubbled with 4% CO_2 + 96% O_2, giving a pH of 7.4. The muscle strips were supramaximally stimulated by 5-sec periods of alternative current via 2 platinum electrodes placed in the bath. The stimulation period was long enough to produce a tension plateau. Time between stimulation was 60 sec. L_{min}, i.e. the length of the unloaded muscle strip, when stimulated, was determined. The length was then increased in steps to and beyond the optimum length (L_0) for active force development. After the experiments the strips were weighed, and cross sectional area at optimum length was calculated assuming a tissue density of 1.05 g per ml. All lengths were expressed in relation to the in situ length of the strip ($L_{in\ situ}$) and to L_0. The whole bladder weight was also recorded. Results are mean ± SE. Student's t-test for unpaired data was used.

Results and discussion. The mean wet weight of the 5 control bladders was 69 ± 5 mg, while that of the 5 decentralized bladders was 334 ± 34 mg; a difference which is significant at a p-level of < 0.005. The length to which the unloaded strip could shorten when stimulated showed a significant difference ($p < 0.01$) between strips of control bladders and those of decentralized bladders; the figures, when related to the length in situ ($L_{min}/L_{in\ situ}$), were 0.30 ± 0.02(5) for control bladders and 0.52 ± 0.06(5) for decentralized bladders. The active length-tension relations for strips of control and decentralized bladders differed markedly when comparisons were based on lengths relative to $L_{in\ situ}$ as can be seen in figure A. The maximal force (P_{max}) developed at optimum length (L_0), expressed in N/cm^2 cross sectional area was, however, about the same for strips of control bladders and for those of decentralized bladders, i.e. 5.9 ± 1.3(5) and 6.8 ± 1.6(5), respectively. When lengths were expressed relative to L_0, the active length-tension curves for both control and decentralized