produced no change in intestinal blood flow (1.21 ± 0.11 ml · min^{-1} · g^{-1}, rotated, vs 1.17 ± 0.10, nonrotated; p = NS). Similarly, the ratio of HAF to PBF was 0.31 when the central liver lobe was not rotated, compared to 0.35 (p = NS) when it was rotated. These results indicate that the experimental conditions necessary for this method do not greatly alter hepatic hemodynamics.

An in vivo method for quantitating $\dot{V}O_2$ in the rat is advantageous because the relationship between O_2 delivery and O_2 extraction can be directly evaluated. For example, an experimental setting that increases $\dot{V}O_2$ should be compensated by an increased O_2 delivery or an increased O_2 extraction. By comparison, previous methods that have evaluated $\dot{V}O_2$ in the rat are liver slice preparations or the isolated perfused liver. Liver slice preparations measure liver respiration[7] and cannot evaluate the O_2 delivery-O_2 extraction relationship. The isolated perfused liver has an O_2 delivery above physiologic levels[8], that cannot change according to O_2 demand as happens in the in vivo preparation. It is acknowledged that $\dot{V}O_2$ in the isolated perfused liver cannot be extrapolated to the in vivo setting[6, 9].

The in vivo method described in this report is particularly applicable for evaluating $\dot{V}O_2$ when chronic pharmacologic administration, for instance, might alter VO_2. In this context, in vivo measurement of $\dot{V}O_2$ is the only method that can adequately evaluate the experimental conditions.

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Regulation of foregut motility in the house cricket, *Acheta domesticus* L.

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Summary. The effects of severance of stomatogastric nerves on the contraction rate of the musculature of the crop of *Acheta domesticus* indicates the existence of a stable myogenic rhythm in the foregut muscularis, which is normally masked by nervous influences.

The foregut of insects generally shows a complex, variable and often continuous activity, apparently independent of the rest of the gut[1]. Though evidence from pharmacological studies[1–6], nerve severance[7–11] and direct stimulation of nerves[3, 12, 13] suggests that nervous influences may be important in the control of the foregut muscularis, the variability of foregut activity and the virtual impossibility of producing truly standardized gut preparations have made definite conclusions difficult. In the present investigation, the nervous influence on the activity of the foregut of *Acheta domesticus* has been examined in isolated gut preparations after nerve severance.

Guts of experimental animals were standardized as far as possible by feeding on an exclusive diet of molar sucrose solution and water for 3 days prior to examination. Entire guts, with stomatogastric nervous system and brain attached, were removed, pinned under slight tension onto Silgard in a petri dish base, and vigorously aerated.

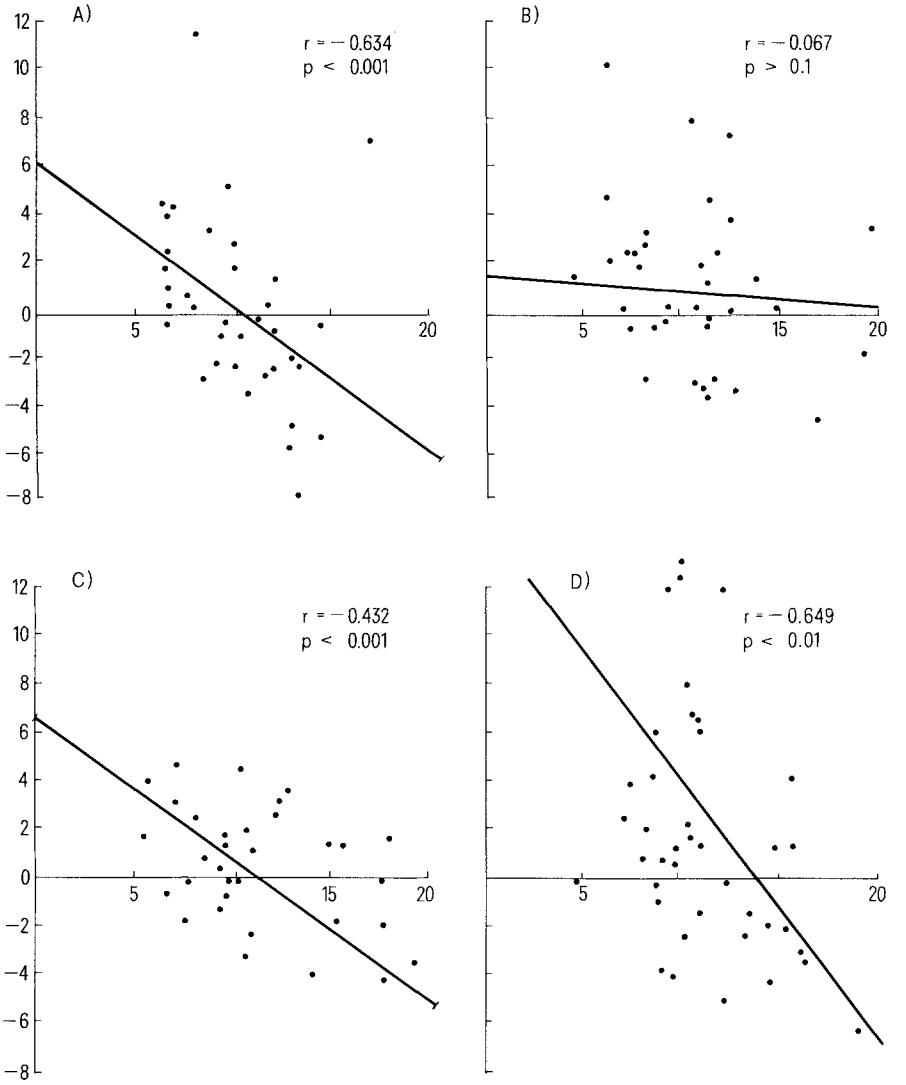
The oesophagus and crop of *Acheta domesticus* show peristaltic waves in both forward and reverse directions. These movements may involve the entire musculature of the region, the ventral surface only, or may be localized, apparently disorganized, areas of movement. The most consistently active region is the ventral area bounded by the 2 oesophageal nerves. Rates of contraction were estimated by selecting a point at about the mid-line of the ventral surface of the crop and counting the waves of contraction passing through it. Even after standardization of isolated guts, the rate of contraction varied from 1 per 2 sec to less than 1 per min.

Experiments to determine the extent of nervous influence on muscular activity in this region took the form of progressive denervation of the isolated gut by sequential severance and extirpation as follows: 1. Frontal connective severance; 2. recurrent nerve severance; 3. hypocerebral ganglion removal; 4. oesophageal nerve removal. Operation 4 leaves the gut entirely denervated except for its own intrinsic neurons.

Frequencies of contraction were often altered by the operations, but in an apparently unpredictable way. However, by plotting the change in the rate of contraction caused by each operation against the rate before surgery, a pattern can be seen (fig.). For all operations except recurrent nerve severance there is a significant correlation between the 2 parameters.

In all cases in which the correlation is significant, the regression line crosses the x-axis at a similar value of preoperative peristaltic rate. This suggests the existence of an underlying stable frequency of contraction in the foregut. Above this frequency, denervation tends to decrease the contraction rate; below it, denervation increases the rate.

By dropping a vertical line through the points at which the



Plots of change in the period of contraction of the crop muscularis as a result of operations on the stomatogastric nervous system against pre-surgery contraction period. *A* Severance of frontal connectives. *B* Severance of the recurrent nerve just posterior to the frontal ganglion, *C* Removal of the hypocerebral ganglion. *D* Removal of the oesophageal nerves. In all cases the vertical axis gives change in the period of contraction in seconds and the horizontal axis represents the period of contraction before surgery, also in sec. A regression line has been fitted in each case. Each point represents the average of the times of 10 pre-surgery and 10 post-surgery contractions in a single preparation.

regression line crosses the x-axis for each graph, the plots of change of frequency against initial frequency can be divided into 4 quadrants, which can be used as the basis for a 2×2 contingency table. This procedure for frontal connective severance gives $\chi^2 = 9.97$, $p < 0.01$, for hypocerebral ganglion removal $\chi^2 = 3.84$, $p < 0.05$, and for oesophageal nerve removal $\chi^2 = 10.29$, $p < 0.01$. The mean value of the suggested stable frequency of contraction, derived from the mean of points of crossing the x-axis for operations yielding significant results, is 1 contraction per 11.57 sec.

Previous experiments studying the effects of nerve severances on foregut operation in insects have yielded rather ambiguous results. Clarke and Grenville[7] and Grenville[9] found that the activity of isolated guts of *Locusta migratoria* and *Schistocerca gregaria* was initially affected in a predictable and repeatable way by severance of stomatogastric nerves, but that more or less normal motility could be regained even after total denervation. Dauterman[8] similarly observed essentially normal motility in the completely denervated foregut of *Blaberus giganteus*. Möhl[11] found that removing or cutting the stomatogastric ganglia and nerves had a destabilizing effect on the operation of the excised crop of *Acheta domesticus*, but in this species, too, occasional specimens showed perfectly normal coordinated activity even when fully denervated. Kamoika[10] found that severance of the recurrent nerve of *Bombyx mori* larvae led to cessation of the normally continuous rhythmical contractions of the pharynx, but that normal motility returned after 20 h. These results suggest that though foregut activity is under the influence of the stomatogastric nervous system it is not exclusively dependent on it.

Möhl[11] has shown that the foregut musculature of *Acheta domesticus* responds directly to applied stretch, in the same way as the rectal longitudinal muscles of the cockroach studied by Cook and Reinecke[14], Nagai[15] and Nagai and Brown[16], and it might be expected that, like these latter muscles, the foregut muscularis possesses a myogenic rhythm. The results of the present investigation suggest the existence of a constant basal frequency for this myogenic rhythm. A fundamental frequency of some sort underlying the muscular activity of the foregut is to be expected. However variable an observed physiological phenomenon, it is a general feature of biological mechanisms that there should be a degree of regulation around a 'normal' level. In the present case the fundamental myogenic frequency of the foregut muscularis is normally masked by nervous activity, and perhaps by other factors. It is unlikely, however, that the value of the basal rate of the myogenic rhythm in the isolated gut bears any close relation to its value in the living animal.

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Release of vasoactive intestinal peptide from rat jejuno-ileum in vitro. Effect of various depolarizing agents[1]

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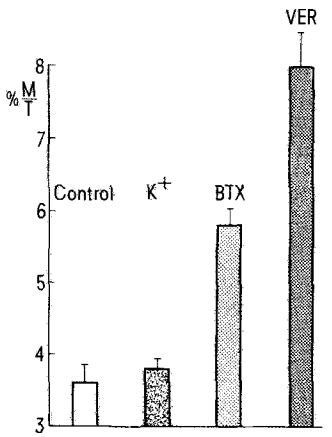
Summary. Vasoactive intestinal peptide (VIP) can be released in vitro from intestinal slices under veratridine and batrachotoxin depolarization, whereas potassium depolarization has no effect. The lack of an effect of potassium observed in this peripheral preparation is different from the positive action described for it in the CNS. The present data suggest that VIP can be released through different mechanisms in the peripheral and central nervous system.

Vasoactive intestinal peptide (VIP), which was first discovered in the gut[2], has also been found in the CNS[3,4]. In the CNS, we have recently shown that depolarizing agents such as potassium, veratridine and batrachotoxin can induce in vitro release of VIP from brain slices[5]. The response to depolarizing stimuli has been considered as one of the criteria to establish a substance as a neurotransmitter. The aim of the present work was to investigate whether those various depolarizing agents were able to induce the release of VIP from intestinal slices in vitro.

Materials and methods. Wistar male rats (250–300 g) were killed by decapitation. The jejuno-ileum was dissected out and washed several times with NaCl 0.24 M EDTA 2.5 mM pH 7.4 in order to remove the epithelial cells, as previously described[6]. The lamina propria was then scraped with a polyethylene tip and cross cut into slices (250 µm) with a McIlwain tissue chopper. Histological controls were performed at each step of the preparation. Microscopic observations showed that there were no more epithelial cells and that the muscular layers were not touched after scraping. The preparation only consisted of the lamina propria, which has been shown to contain VIP nerve terminals[7]. Slices were washed several times until a clear supernatant was obtained in cold Krebs Ringer Bicarbonate (KRB) buffer (pH. 7.4) containing (mM): NaCl 118, KCl 5, $CaCl_2$ 2.5; KH_2PO_4 1.2, $NaHCO_3$ 25, Glucose 10, Bacitracin 2×10^{-5} M and trasylol 500 kIU/ml. Slices were preincubated in KRB buffer under 95% O_2 - 5% CO_2 during 30 min at 37 °C. The incubation was started by addition of a lamina propria preparation (5 mg tissue in 200 µl KRB) to polyethylene tubes containing the various substances being tested. The incubation was then carried out for 10 min at 37 °C and ended by centrifugation in an Eppendorf microcentrifuge at 10,000 × g for 2 min. The slices were immediately extracted with 0.1 N HCl, sonicated for 15 sec and samples were frozen until assay. VIP content was assayed both in the supernatant and the tissue extract using a specific radioimmunoassay for the peptide[8]. VIP release was expressed as the percentage of VIP secreted in the medium over VIP content in the tissue.

Results and discussion. The effect of potassium (K^+), batrachotoxin (BTX) and veratridine (VER) on the release of VIP from the lamina propria of intestinal villi is shown on the figure. The basal amount of VIP released in the medium represents 3.6 ± 0.3% of the total content in the tissue. BTX (1 µM) and VER (50 µM) induced a significant release of VIP ($p < 0.01$ vs control) which represented 1.6- and 2.2-fold increases of the basal level, respectively. On the contrary, K^+ (56 mM) does not significantly increase the release of VIP from the intestinal preparation.

Increasing extracellular K^+ concentration in the incubation medium does not seem to influence the basal release of VIP from slices of the lamina propria. This lack of effect is not due to the absence of VIP nerve terminals in this region, since both VER and BTX increase the secretion of the peptide under similar conditions. Moreover, immunocytochemical data have shown abundant VIP-containing cell bodies and fibers in the lamina propria of different species



Effect of potassium 56 mM (K^+), batrachotoxin 1 µM (BTX) and veratridine 50 µM (VER) on the release of VIP from gut lamina propria. The release of VIP is expressed as the percentage of VIP secreted in the medium (M) over the amount of VIP present in the tissue (T). The data are mean ± SEM of 8 determinations and are a representative example of 6 different experiments. Data from the other experiments are similar, and show no significant differences between groups, as found by analysis of variance.