Number of units receiving excitation from the posterior contralateral connectives at different levels of the ventral nerve cord on photic stimulation of the 6th abdominal ganglion.

Direction of crossing over of the photic input		No. of preparations examined	Mean No. of units (Values corrected to the nearest whole number)	Maximum No. of units observed in a preparation
From	To			
Connectives				
Left 5–4	Right 4–3	14	6	8
Left 4–3	Right 3–2	14	5	7
Left 3–2	Right 2–1	18	3	6
Left 2–1	Right 1-SOG[a]	16	2	4
Right 5–4	Left 4–3	14	5	8
Right 4–3	Left 3–2	14	6	8
Right 3–2	Left 2–1	16	3	6
Right 2–1	Left 1-SOG[a]	14	4	6
Left 5–4 to Right 4–3 to Left 3–2		12	2	5
Right 5–4 to Left 4–3 to Right 3–2		12	3	4

[a] Suboesophageal ganglion.

cross over from one side of the nerve cord to the other. Such a distribution of fibres, whereby they travel across a ganglion and enter the next contralateral connective, has been described in the nerve cord of scorpion[4]. But, in the present examples, the responding units in the two contralateral connectives did not resemble each other in spike characteristics, and there was no strict correspondance between the activity in the two connectives. This suggested that the units in the anterior contralateral connectives are synaptically activated in the intermediate ganglia even though the alternate possibility in other cases is not altogether ruled out. Similar excitation of contralateral units by tactile afferents and divergence of tactile input has been demonstrated earlier in scorpion[5].

The decrease in the frequency of contralateral excitation anteriorly indicates that there may be dropping off of the sensory input from the metasoma. It may also mean that there is greater convergence of units anteriorly. This is further supported by the anatomical studies, wherein the number of fibres in the connectives were found to decrease in the postero-anterior direction in the ventral nerve cord of scorpion[4].

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Alternative Methods of Animal Sacrifice: The Effect on Intestinal Function in vitro

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Summary. The total fluid uptake of everted sacs of rat jejunum was compared in animals killed by stunning and decapitation, or anaesthetised with ether. Fluid transport was significantly higher in the tissue prepared from stunned and decapitated animals. It is suggested that etherization may have adverse effects on the physiological viability of subsequently isolated tissues.

Despite the widespread use of in vitro intestinal preparations there appears as yet to be little standardization of technique. The short study described herein was undertaken in order to decide between two alternative methods of animal sacrifice, etherization, and stunning and decapitation, prior to the preparation of everted sacs. Fluid absorption was chosen as a convenient index of structural viability as it is thought to occur as a corollary of active solute transport at the mucosal surface[2].

Methods. Male Wistar rats of 180–210 g body weight were allowed water ad libitum but were deprived of food (Heygates 41B) for about 18 h overnight before use. Animals were sacrificed by 2 methods.

1. *Etherization.* The rat was placed on the metal grid of a glass vacuum-desiccator, the lower section of which was filled with a quantity of cotton wool soaked in diethyl ether. As soon as the animal lost consciousness it was removed from the killing jar, the abdomen was opened with a mid-line incision and the entire small intestine was removed by severing it at the duodenal-jejunal flexure, and at the ileo-caecal junction. The animal was then killed by decapitation.

2. *Stunning and decapitation.* The rat was placed gently on the surface of a laboratory stool with a minimum of disturbance. After a few sec the animals was swung suddenly by the tail and stunned with a single blow against

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the stool. The spine was then severed at the base of the skull, and the intestine was removed as previously described.

The exsected intestine was immediately transferred to a beaker containing 50 ml of oxygenated saline (0.9% NaCl) at 0–4 °C, and the lumen was flushed through with the same solution delivered from a wash-bottle via the jejunal opening. The material was then everted on a surgical quality stainless steel rod (1.5 mm diameter, 40 cm length) and 4 everted sacs were prepared from the first 40 cm of the proximal jejunum[3]. Sacs were placed at random into 25 ml Erlenmeyer flasks and incubated for 30 min in 5.0 ml Krebs phosphate Ringer containing 28 m*M* glucose. The flasks were gassed continuously with 5% CO_2 in O_2 and maintained at 37 °C in a shaking incubator running at 100 oscillations per min. Total water transport was assessed by weighing the sacs empty and filled, prior to, and after incubation.

Results. The total fluid transport for each animal, expressed as ml per g initial wet weight per h, was derived as the mean value for 4 sacs, together with the standard error of the mean. The value for 7 animals sacrificed by ether anaesthesia was 0.37 ± 0.05 compared with 0.64 ± 0.06 for the 11 stunned and decapitated animals ($p < 0.01$).

Discussion. The removal of tissue from anaesthetized rather than freshly killed animals is usually justified on the grounds that it minimizes the period of arrested circulation prior to the isolation of the tissue and reduces the effects of shock induced by the trauma of sacrifice. Indeed some workers stress the importance of perfusing the intestine whilst the blood supply is still intact[4]. An obvious disadvantage of anaesthetics is their unknown effects on the metabolism of the intestine, and the necessity of working with tissue which has been exposed to unknown levels of potentially toxic materials.

In the present study it has been shown that the fluid uptake capacity of the isolated intestine is significantly lower in etherized as compared to stunned rats. The reasons for this remain obscure, but a reduction in glycolysis has been previously reported in everted sacs prepared from animals sacrificed with ether, and it seems possible that the effect is due to a direct toxic inhibition of energy-dependent processes in the mucosa[5].

It would be wrong to infer from the present results that stunning and decapitation is necessarily the ideal method of animal sacrifice, but it is suggested that minor aspects of the experimental technique used in this sort of work may have far-reaching effects on the viability of the tissue under study and this should be borne in mind when planning new research or comparing the accounts of other authors.

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Concerning the Ionic Basis of Presynaptic Inhibition

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Summary. The superfused rat cuneate nucleus has been used to investigate the sensitivity of primary afferent terminals and of evoked primary afferent depolarization (PAD) to alterations in extracellular K^+ and Cl^- ion levels. Results indicate that PAD is caused by an efflux of Cl^- from primary afferent terminals rather than by an increase in extracellular K^+.

Although it is generally accepted that depolarization of primary afferent terminals accompanies presynaptic inhibition in most vertebrate species, it is uncertain how this depolarization is brought about. The original suggestion[2] that primary afferent depolarization (PAD) is a secondary phenomenon dependent on an increase in extracellular potassium levels around afferent terminals has received support from recent studies using potassium-sensitive microelectrodes[3,4]. Another idea, strongly supported by electrophysiological and pharmacological observations, is that presynaptic inhibition is a primary synaptic event and that PAD is brought about by the direct action on afferent terminals of the presynaptic inhibitory transmitter, the amino acid GABA (γ-amino-*n*-butyric acid)[5,6]. Since the best established membrane actions of GABA are mediated by chloride ions rather than by potassium[7], the experiments reported here were designed to test the two hypotheses by investigating the sensitivity of primary afferent terminals and of evoked PAD to alterations in the extracellular levels of both potassium and chloride ions.

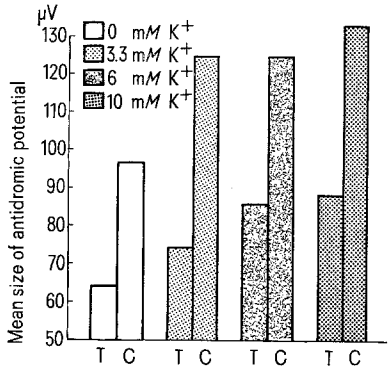

Fig. 1. The effect of increasing the potassium content of the cuneate superfusate in stages from 0 to 10 m*M* on both test (T) and conditioned (C) antidromic potential height. Testing and conditioning stimulus strengths remained constant throughout with conditioning stimulus strength supramaximal and interstimulus interval 15 msec.

Materials and methods. The exposed cuneate nucleus in chloralose-urethane anaesthetized rats was superfused

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