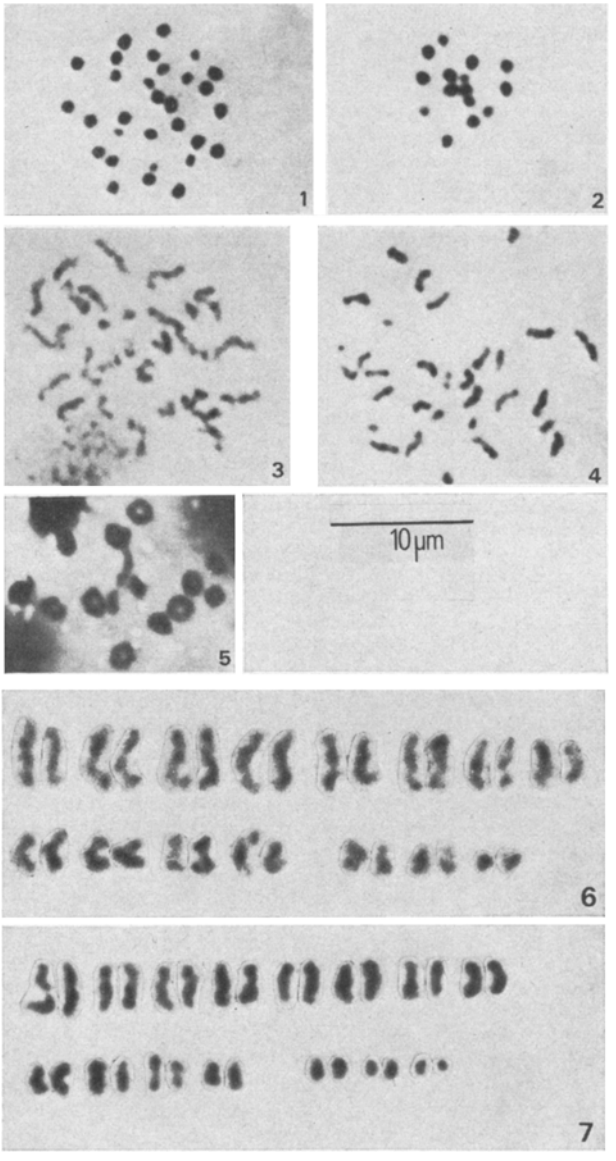

Fig. 1. Spermatogonial metaphase from an untreated larva.
Fig. 2. Metaphase II from an untreated larva.
Fig. 3 and 4. Spermatogonial metaphases from treated larvae.
Fig. 5. Metaphase I from a treated larva.
Fig. 6 and 7. Karyograms made from figures 3 and 4.

karyotypes and their evolutionary significance, the nature of the kinetochore, bivalent configuration, etc. But the morphological details of the karyotypes that would help to analyse the elements individually are still lacking in this group.

Miller and Miller[3] obtained elongated chromosomes in a lepidopteran species, in which the elements showed a nice beaded appearance. However, these chromosomes could not be arranged in a karyogram for making a comparison among the elements*. We have been successful for the first time in obtaining in Pieris brassicae elongated chromosomes that can be characterized on the basis of their length. This species was selected because it has fewer chromosomes than others. Chromosomal preparations were made by using an air-drying technique after injecting the prepupal larvae with 0.01 ml of 0.1% colchicine solution 2 h before dissection. Preparations were also made without the treatment with colchicine. In both the cases, the testicular tissue was suspended in 1.0% sodium citrate solution which was replaced after 20 min by acetic alcohol (1:3) through centrifugation. After resuspending the material and centrifugation, the old fixative was changed by a small amount of the new one. The material was again stirred into suspension which, after 30 min, was spread on clean slides and air-dried. These slides were stained in Giemsa solution.

In the case of uninjected larvae, spermatogonial metaphases revealed 30 rounded chromosomes of the usual lepidopteran type (figure 1). Metaphase II plates possessed 15 elements of the same nature (figure 2). In the treated material, however, the spermatogonial metaphases revealed 30 elongated chromosomes showing varying lengths (figures 3 and 4). From these plates it was possible to cut out the elements and characterize them individually on the basis of their lengths (figures 6 and 7). The 2 karyograms given here show in each case 8 pairs of large elongated chromosomes, 4 pairs of medium-sized elements and 3 pairs of smaller ones. No elements could be designated as the sex chromosomes. However, there is a lone heteromorphic pair in the group of medium-sized elements. Some chromosomes show hooked appearance, which is not due to any localized kinetochores. In fact, the inability of colchicine to separate the chromatids confirms the holokinetic nature of the chromosomes. Metaphase I shows 15 bivalent with clear chiasmata (figure 5). The numerical count made by earlier workers[4–6] is confirmed in this analysis. It is believed that further studies on this pattern may be more useful in the lepidopteran species for determining any evolutionary trends and chromosomal changes in their karyotypes.

3 L. D. Miller and S. M. Miller, Science *152*, 529 (1966).
4 N. K. Beliajeff, Z. indukt. Abstamm.- u. Vererb-Lehre *54*, 369 (1930).
5 H. Federley, Hereditas *24*, 397 (1938).
6 Z. Lorkovic, Chromosoma *2*, 155 (1941).
* As this paper was in press, we saw a similar report of T. R. L. Bigger (Cytologia *40*, 713, 1975), but describing P. brassicae chromosomes as monocentric. This is not confirmed by us here.

Synapsing pathways through the guinea-pig inferior mesenteric ganglion

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Summary. All 4 groups of nerves connecting with the guinea-pig inferior mesenteric ganglion contain synaptic inputs which activate postganglionic activity in all groups except the lumbar splanchnic nerves.

The guinea-pig inferior mesenteric ganglion (IMG) receives a multiple input from lumbar splanchnic nerves (LSN), hypogastric nerves (HN), ascending mesenteric nerve (AMN) and colonic nerves (CN). Intracellular recordings have shown that most ganglion cells receive several inputs from each of these sources with up to 40 fibres synapsing

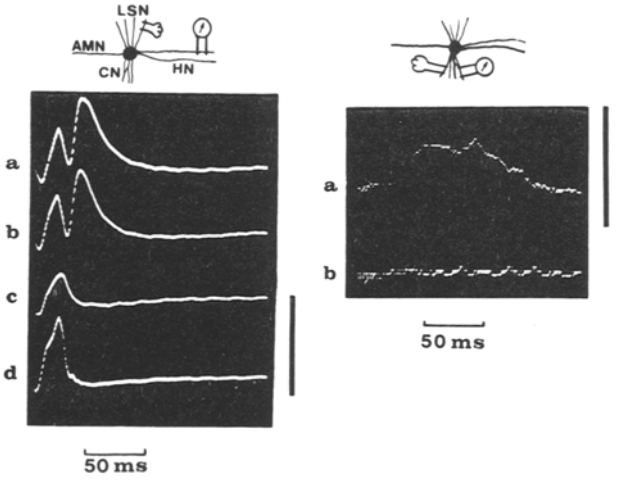
on to each cell[1]. We have used extracellular recording techniques to demonstrate synapsing and possible direct, or non-synapsing, pathways through the ganglion.

Methods. Young male guinea-pigs (200–400 g) were anaesthetized with urethane (1.8–2.4 g kg^{-1} i.p.). The abdomen was opened and the skin edges sutured to a metal ring to form a pool which was filled with liquid paraffin. The intestines were pulled out of the pool and wrapped in a moist gauze swab. The terminal loop of the large bowel was then manipulated within the pool to expose the IMG and connecting nerves. Overlying mesentery and adipose tissue were cut away. The ganglion was decentralized in most experiments though not necessarily at the start. The nerves selected for stimulating and recording were lifted on to bipolar silver or platinum electrodes. The recorded potentials were viewed directly and also relayed to a signal averager (Palmer 8137) from which all the records were made. Nicotine (1%, at pH 7.4 applied topically to the IMG) was used to block synapsing pathways. When nicotine was applied to individual nerve trunks, such as the hypogastrics, there was no change in the compound action potential; not even in the C fibre component. Blockage of activity by nicotine applied to the ganglion was taken therefore to represent blockage of synaptic transmission only. The results obtained by this technique were confirmed, in many cases, by injecting hexamethonium (2–5 mg kg^{-1} i.v.) or by using repetitive stimulation at 20 Hz to block synaptically evoked potentials.

Summary of results

Position of recording electrodes	Position of stimulating electrodes			
	AMN	CN	HN	LSN
AMN	S a c 2 2	SR a b 2 1	SR a c 3 1	SR a c 2 2
CN	SR a 2	S a b c 4 1 3	SR a b c 3 1 2	SR a c 4 3
HN	SR a c 6 1	SR a c 3 3	S a b c 6 2 4	SR a b c 5 2 5
LSN	R a b c 2 1 1	R a b c 2 1 1	R a 2	X 3

S, Synapsing pathways only; NS, resistant pathways only; SR, synapsing and resistant pathways identified; X, no activity recorded; *a* identified using nicotine, *b* identified using hexamethonium, *c* identified using high frequency blockade. Number of individual experiments shown below each code letter.



Results of 2 experiments. Left: Effect of stimulating LSN and recording from HN. Arrangement of stimulating and recording electrodes shown above trace. For labelling see text. Traces *a* and *b*, control responses. Traces *c* and *d*, responses after topical application of 1% nicotine to IMG. Right: Effect of stimulating CN and recording from CN. *a* Control responses with LSN intact. On cutting LSN the late wave disappears leaving only the early peak. *b* Response after topical 1% nicotine, LSN still intact. Vertical bars: 50 μV.

Results. The figure illustrates 2 typical observations. In one example, the activity evoked in a colonic nerve by stimulating other colonic nerves was blocked by topical nicotine, thereby demonstrating that all the activity was reflexly excited. In this particular preparation only the early part of the wave persisted after decentralization, and the slow late wave therefore represents a spinal component and the early wave the peripheral ganglionic reflex. In the other example, recording from a hypogastric nerve and stimulating a lumbar splanchnic nerve, the response consisted of 2 waves. The late wave was blocked by nicotine while the early wave remained unaltered. The table lists the pathways identified and indicates the criteria used to establish their existence. Where 2 or more methods of blocking synaptic transmission were used, identical results were obtained in each case. The resistant pathways are possibly non-synapsing though they may include resistant synapsing pathways. The lumbar splanchnic nerves contain preganglionic fibres synapsing with inferior mesenteric ganglion cells as well as resistant pathways. However no post-ganglionic fibres from IMG cells, activated by any of the inputs to the ganglion, were found in the lumbar splanchnic nerves. Only synapsing pathways pass to and from the ganglion in the same nerve or group of nerves. All other possible routes are a mixture of synapsing and resistant (and therefore possibly non-synapsing) pathways.

Discussion. The present study confirms that all 4 groups of nerves contain preganglionic or centripetal fibres making synaptic connections within the IMG. In addition, all nerves except the LSN contain post-ganglionic fibres which can be excited reflexly by stimulation of any of the 4 groups of inputs. A similar general arrangement has been suggested before, based on the combined results of a number of individual workers, using several different species[2]. A complete analysis of the functional pathways in a single species has not been reported. This functional organization provides a basis for a number of peripheral sympathetic reflexes as well as for possible interactions between the IMG and other peripheral sympathetic ganglia.

1 P. J. Crowcroft and J. H. Szurszerski, J. Physiol. *212*, 421 (1971).
2 V. I. Skok, in: Physiology of Autonomic Ganglia, chapter 2, p. 41. Igaku Shoin Ltd, Tokyo.