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REFLEX FIRING IN THE LUMBAR SYMPATHETIC OUTFLOW TO ACTIVATION OF VESICAL AFFERENT FIBRES*

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SUMMARY

1. Activation of vesical afferent fibres in the A$\gamma\delta$ range by electrical stimulation of the pelvic nerve or by bladder distension elicited reflex firing in hypogastric nerves and in preganglionic nerves to the inferior mesenteric ganglion.

2. The multisynaptic reflex was present in cats with an intact spinal cord and in acute and chronic spinal animals (transections at T10–T12). The reflex pathway was partially crossed in the sacral cord, and in the periphery at the level of the inferior mesenteric ganglia. In contrast, an inhibitory response to raised intravesical pressure was mediated by a supraspinal inhibitory mechanism which was activated in parallel with the micturition reflex.

3. Since enhancement as well as depression of sympathetic firing accompanied reflex micturition, it is concluded that at least two distinct populations of lumbar preganglionic neurones are responsive to vesical afferent activity: one population being excited, the other depressed, during micturition. The latter population may be involved in an inhibitory feed-back mechanism on to the bladder.

INTRODUCTION

The urinary bladder of the cat is innervated by efferent autonomic fibres in the pelvic and hypogastric nerves (Langley & Anderson, 1895, 1896). The pelvic (parasympathetic) innervation is the principle excitatory input to the bladder; and its integrity is essential for the normal performance of micturition (see reviews by Langworthy, Kolb & Lewis, 1940; Kuru, 1965). The hypogastric (sympathetic) innervation, on the other hand, is primarily inhibitory; but its importance in the regulation of bladder activity is not clearly established (see Langworthy *et al.* 1940).

* All experiments described in this paper were carried out in the United States.

To elucidate the role of the sympathetic nerves in bladder function, the reflex changes in sympathetic firing following activation of afferent fibres from the bladder have been recorded and it has been shown that central reflex mechanisms exist by which vesical afferent activity can influence the lumbar sympathetic outflow. In agreement with Edvardsen (1968*a*) it is concluded that these mechanisms are organized primarily at the spinal level. The reflexes may subserve several functions, one of which may be an inhibitory feed-back mechanism on to the bladder (Edvardsen, 1968*a*, *b*). A preliminary account of these observations has been published (Lalley, de Groat & McLain, 1971).

METHODS

Experiments were performed on fifty cats (both male and female) anaesthetized with chloralose (50–70 mg/kg, I.V.). In four animals the spinal cord had been transected at the lower thoracic level, 8–31 days before the experiment. Anaesthesia was induced in all animals with halothane. Following intubation of the trachea, the urinary bladder and its neural innervation were exposed through a mid line abdominal incision. Hypogastric nerves (bilaterally), colonic nerves and several preganglionic nerves to the inferior mesenteric ganglia were dissected free from underlying connective tissue. The nerves were either left intact or sectioned and crushed distally for monophasic recording. Branches of the pelvic nerves were isolated 1–3 cm from the neck of the bladder and sectioned or crushed peripherally. In a few experiments, the superior cervical ganglion and the post-ganglionic sympathetic nerves to the external carotid artery and the cervical spinal roots were also isolated and prepared for recording. In three animals, baroreceptor afferents in the carotid sinus nerve were activated by distension of the carotid sinus. A cannula was inserted into the common carotid artery after occlusion of all other vessels from the sinus region. Sinus pressure, which was measured via a cannula in the lingual artery, was raised by injecting warm physiological saline into the carotid cannula. In these experiments, the vagus and aortic depressor nerves were sectioned bilaterally.

The urinary bladder was cannulated by one of two different methods. In some cats (primarily females) a polyethylene tube (inside diameter, 2 mm) was introduced into the urethra either through the external orifice or through an incision in the urethra and passed into the bladder. The cannula was secured in place by a ligature around the urethra. In other cats, an incision was made in the fundus of the bladder and a thin-walled rubber condom mounted on the end of a flexible tube (inside diameter, 6·4 mm) was inserted into the lumen (de Groat & Ryall, 1968). The cannula was filled with physiological saline solution and connected to a pressure transducer to record the pressure within the bladder. The cannula could also be connected via a three-way stopcock to a reservoir of large surface area, the height of which could be adjusted to maintain a constant pressure in the bladder over a range of 0–60 cm H_2O. In some experiments, a laminectomy was performed from L 1 to S 3 and the animal was placed on its side to provide access to both the spinal cord and the abdominal cavity. Exposed areas were covered with warmed paraffin oil.

Isolated nerves were mounted on bipolar silver electrodes for stimulation and recording. Stimulation was produced by rectangular pulses of 0·01–0·2 msec duration at varying frequencies and intensities. Action potentials were displayed on an oscilloscope and photographed on 35 mm film. Potentials were also averaged on a Computer of Average Transients (CAT), the output of which was then plotted on an

X–Y paper recorder. The magnitude of an averaged potential was measured with a planimeter as the area under the evoked response.

The majority of animals were paralysed with gallamine triethiode and artificially respired. Experience with unparalysed preparations indicated that the dose of chloralose administered was sufficient to produce surgical anaesthesia for the duration of the experiments. In addition during the experiments depth of anaesthesia was often checked by discontinuing the administration of gallamine and allowing the animal to recover from the paralysis. End tidal CO_2, which was monitored continously with a Beckman Medical Gas Analyzer, was maintained at approximately 4% by varying the rate and depth of respiration. Systemic blood pressure was measured from the carotid or femoral arteries with a strain gauge pressure transducer. The animal's temperature was maintained at 36–38° C with the aid of a heating pad. End tidal CO_2, blood pressure, bladder pressure and frequency of neuronal firing were displayed on a rectilinear multichannel paper recorder.

In those animals in which the spinal cord was partially transected, histological sections of the cord were prepared to determine the extent of the lesion. Spinal cords were removed, fixed in 10% formal saline and then sectioned at 50 μm. The sections were stained with luxol fast blue and cresyl violet or with thionin.

RESULTS

Background sympathetic firing. Multifibre recordings from the hypogastric nerves (HGN) or from preganglionic nerves to the inferior mesenteric ganglion (IMG) revealed different patterns of spontaneous sympathetic firing. The most common type of activity was an irregular discharge having no obvious temporal relationship with the respiratory or cardiac cycles. Less frequently, firing was correlated with respiration. In paralysed, artificially respired preparations, this type of activity was characterized by increased firing during expiration. Superimposed upon this rhythm, there sometimes occurred a smaller modulation in firing which corresponded with the cardiac cycle. In some animals a marked respiratory or cardiac rhythm could be recorded on one preganglionic nerve and yet be undetectable on an adjacent nerve to the same ganglion. Irrespective of the underlying discharge pattern, the most striking alteration in lumbar sympathetic activity occurred during contractions of the urinary bladder (Fig. 1).

The correlation between spontaneous bladder contractions and sympathetic firing. Rhythmic contractions were observed when the bladder was distended and maintained under constant volume conditions. This type of bladder activity was dependent upon firing in the parasympathetic efferent pathway (de Groat & Ryall, 1969) and was markedly reduced or abolished by transection of the spinal cord in the lower thoracic region (Fig. 1*B*). In most experiments (75%) the contractions were accompanied by an increase in spontaneous efferent firing in the HGN and in preganglionic fibres to the IMG (Figs. 1 and 2). The sympathetic firing commenced during the

initial rise in intravesical pressure (Fig. 2*D*, *E*) and commonly reached a maximum before the peak of a bladder contraction. This relationship was observed in animals with intact spinal cords (Figs. 1 and 2; fourteen experiments) and in animals where the cord had been completely transected above the lumbar sympathetic outflow (T 10–T 12), either during the experiment (acute spinal, Fig. 1*A*) or 1–4 weeks before the experiment (chronic spinal, four experiments, Fig. 2*D*, *E*). The firing was not observed after bilateral transection of the pelvic nerves or transection of the cord at

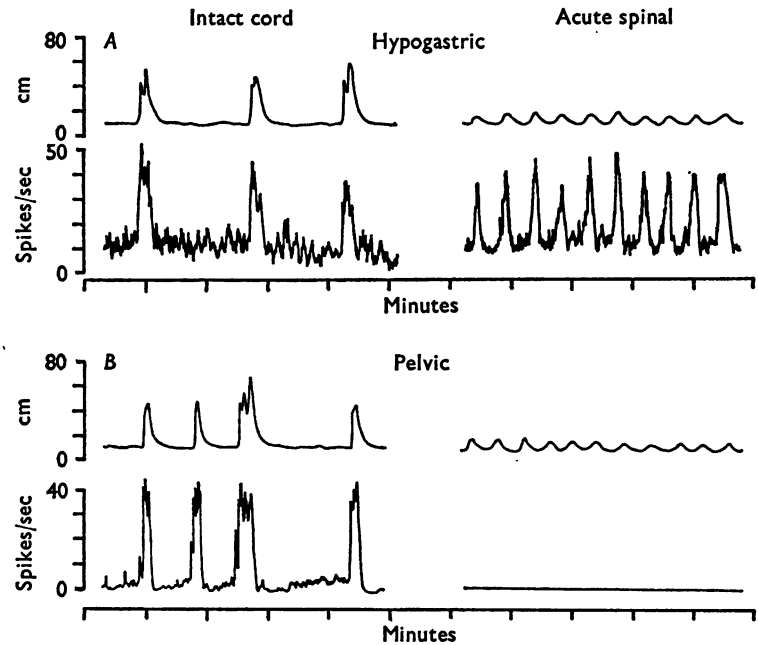

Fig. 1. Relationship of isovolumetric bladder contractions to spontaneous efferent firing on hypogastric (*A*) and pelvic (*B*) nerves. Responses in the left hand column of *A* and *B* were recorded with the spinal cord intact, those in the right hand column were obtained in the same animal 10 min after transecting the spinal cord at T 11. Ordinates: bladder pressure in cm H_2O (upper records), spontaneous firing in spikes/sec (lower records). Cat was anaesthetized with chloralose.

the lower lumbar levels. Thus, the afferent pathway for the reflex must be located in the pelvic nerves; and the reflex must be completely organized within the lumbo-sacral cord. In contrast to the vesico-sympathetic reflex, reflex firing in parasympathetic fibres (pelvic) to the bladder was abolished by acute transection of the spinal cord (Fig. 1*B*).

Sympathetic preganglionic units fired at peak frequencies of 2–12 impulses/sec during bladder contractions (Fig. 2). Between contractions

some fibres were silent, whereas others fired at relatively regular rates of 0·5–2 impulses/sec. Immediately following a bladder contraction or during the descending phase of a contraction the firing of some units transiently decreased below control levels. A decrease in spontaneous sympathetic firing was observed during bladder contractions in three of twenty experi-

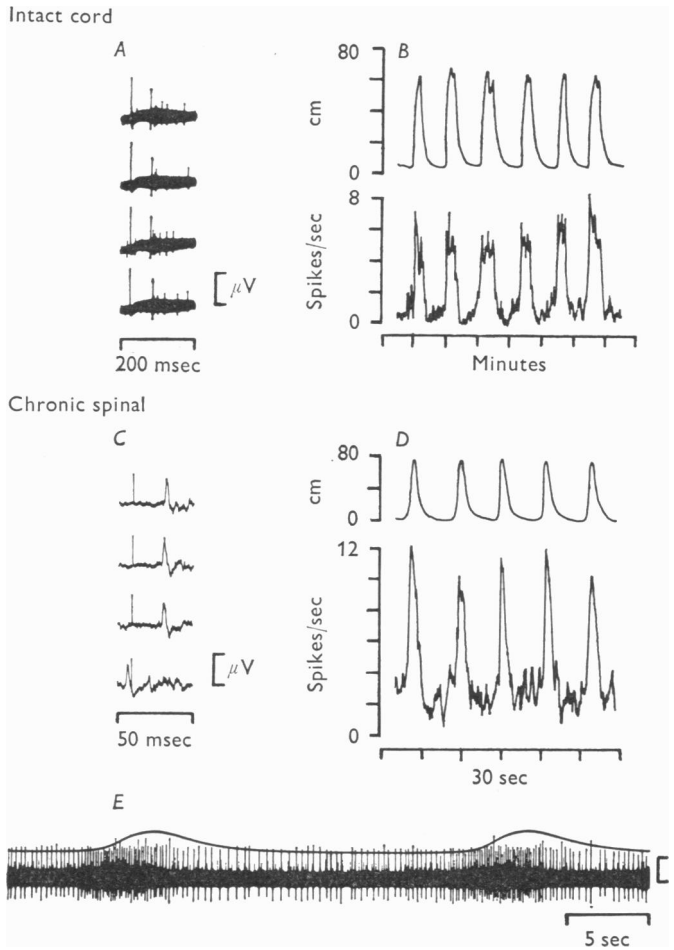

Fig. 2. Single unit responses on lumbar preganglionic nerves to electrical stimulation (0·5 c/s) of pelvic afferent fibres (*A*, *C*) or during isovolumetric bladder contractions (*B*, *D*, *E*). Records *A* and *B* were obtained from the same unit in a cat with an intact spinal cord. Records *C*, *D*, and *E* were from a similar unit in a chronic spinal animal. *B* and *D*, upper records, bladder pressure in cm H_2O; lower records, frequency of single unit firing in spikes/sec. *E*, upper record, bladder pressure, lower record, firing of a preganglionic fibre. Vertical calibrations: *A*, 50 μV. *C*, 100 μV. *E*, 80 cm H_2O (upper tracing), 80 μV (lower tracing), negativity upward. Cat was anaesthetized with chloralose.

ments (Fig. 3). Since these inhibitory responses were observed infrequently their importance might be questioned. However, Bradley & Teague (1969) have obtained inhibitory responses more consistently in unanaesthetized decorticate cats. The presence of the inhibition might be dependent, therefore, on the type of preparation employed.

One preganglionic unit was encountered which was inhibited during bladder contractions (Fig. 4). The unit usually exhibited a complete suppression of firing during the rising phase of a bladder contraction (Fig. 4*C*, *D*, *F*), a partial recovery at the peak of a contraction, and secondary depression followed by an enhancement of activity during the decline in

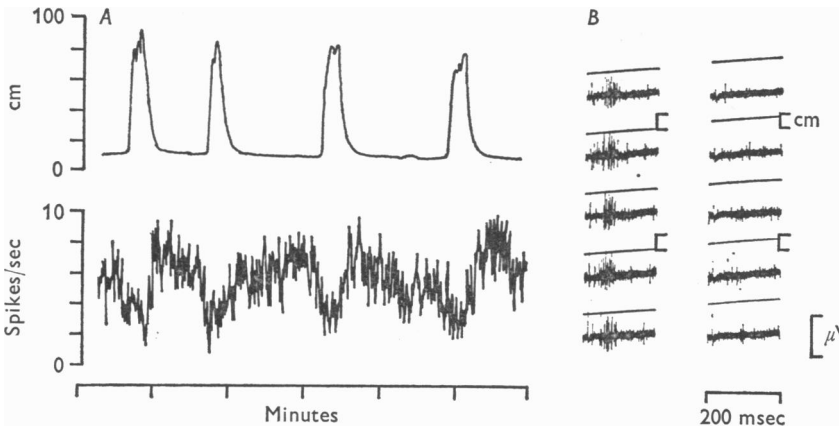

Fig. 3. *A*, inhibition of spontaneous preganglionic sympathetic firing during isovolumetric contractions of the bladder. Upper record, bladder pressure in cm H_2O; lower record, frequency of spontaneous preganglionic firing in spikes/sec. *B*, inhibition of the pelvic to preganglionic sympathetic reflex during isovolumetric contractions of the bladder. Upper tracing in each record is bladder pressure and lower tracing is the preganglionic reflex evoked by a single stimulus to the pelvic nerve. Records on the left were obtained between bladder contractions and on the right during a bladder contraction. Vertical calibrations in *B* are 100 cm H_2O and 90 μV, negativity upward. Cat was anaesthetized with chloralose.

bladder pressure. The unit was fired at 60 msec latency by electrical stimulation of vesical afferent fibres (Fig. 4*A*). Interestingly, Bradley & Teague (1969) also noted in their experiments a transient increase in HGN-firing following the period of inhibition.

Several units were unaffected by bladder activity. Two of these units had high (7–12 impulses/sec) and very regular firing rates unrelated to the respiratory or cardiac cycles. Another unit fired in time with the cardiac cycle and was inhibited during distension of the carotid sinus. Spontaneous firing on an adjacent preganglionic nerve in the same animal was resistant

to carotid distension, but was enhanced during bladder contractions. In another experiment, multiunit firing, in the same preganglionic nerve, was enhanced during bladder activity and depressed during baroreceptor activation.

Rhythmic discharges in time with bladder contractions were not observed on the colonic nerves (i.e. post-ganglionic branches from the inferior mesen-

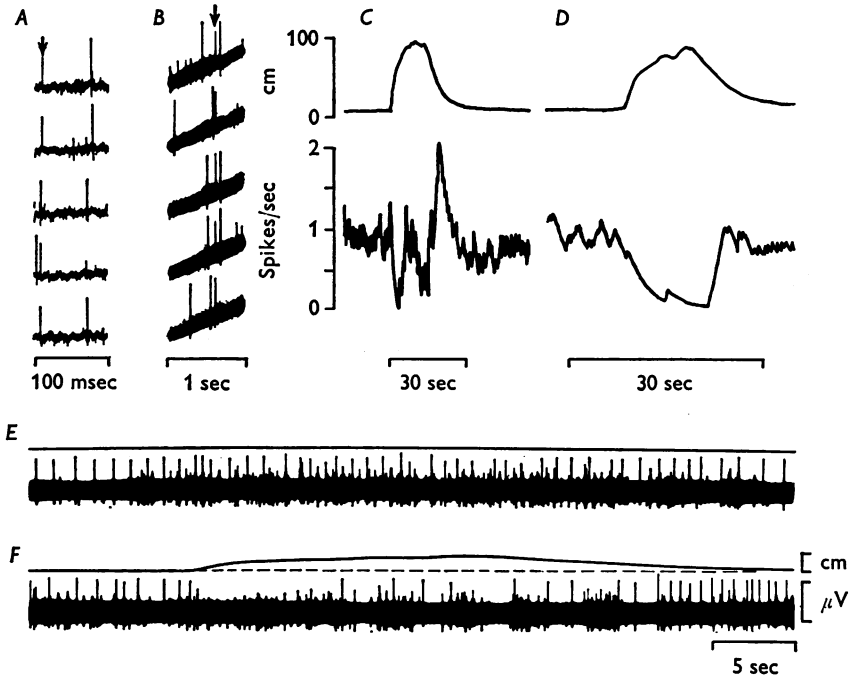

Fig. 4. *A* and *B*, reflex firing in a preganglionic sympathetic unit in response to single shocks to the pelvic nerve in a cat anaesthetized with chloralose. Note depression of evoked response by spontaneously occurring action potentials. Stimulus artifacts marked by arrows. *C*, *D*, *E* and *F*, inhibition of spontaneous preganglionic firing during isovolumetric bladder contractions. Same preganglionic unit as in *A* and *B*. *C* and *D*, upper tracing is bladder pressure in cm H_2O, lower tracing is frequency of firing in spikes/sec. Time constant of rate-meter was 0·5 sec in *C* and 3 sec in *D*. *E* and *F*, upper tracing is bladder pressure and lower tracing is spontaneous preganglionic firing. Vertical calibrations in *E* and *F* are 100 cm H_2O and 80 μV, negativity upward. The latter calibration also applies to records *A* and *B*.

teric ganglion to the colon) or on post-ganglionic nerves emanating from the superior cervical ganglion. However, in twelve of twenty-seven animals, bladder contractions were accompanied by a rise in systemic blood pressure (Fig. 5) indicating activation of vasoconstrictor fibres.

The effect of bladder distension on sympathetic firing. When intravesical pressure was raised artifically by injecting small volumes of fluid into the

bladder, or by subjecting the bladder to a constant pressure from an elevated fluid reservoir, spontaneous firing on the HGNs and preganglionic nerves was enhanced (fourteen experiments). This response was observed in animals with an intact spinal cord, and also in acute spinal and chronic spinal animals. The increased firing was maintained for as long as the bladder was distended (1–3 min) and returned to control levels in parallel with bladder pressure. The lowest bladder pressures to evoke a detectable response ranged from 10 to 30 cm H_2O, whereas maximal responses were

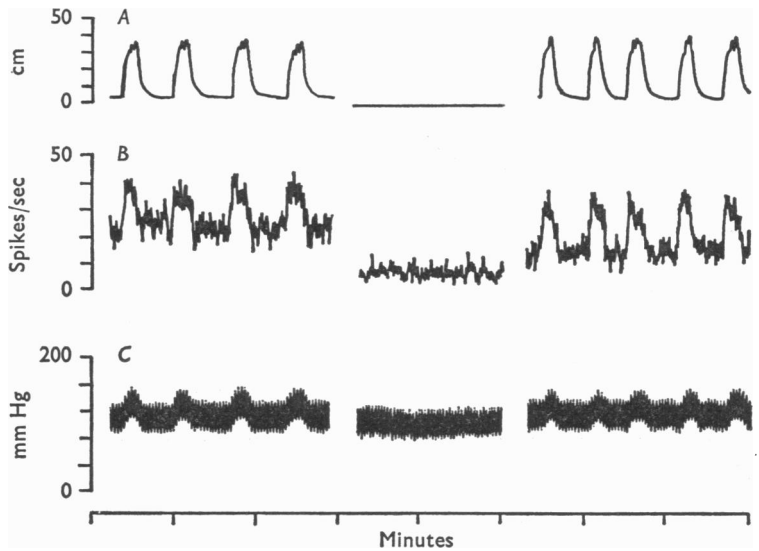

Fig. 5. Isovolumetric contractions of the bladder (*A*) and accompanying increases in spontaneous lumbar preganglionic firing (*B*) and blood pressure (*C*) in a cat under chloralose anaesthesia. Note in middle column the absence of phasic increases in preganglionic firing and blood pressure when bladder was evacuated. Ordinates: *A*, bladder pressure in cm H_2O; *B*, frequency of lumbar preganglionic firing in spikes/sec; *C*, blood pressure in mm Hg.

obtained at pressures of 30–40 cm H_2O. Firing on colonic nerves was enhanced in one of six experiments during bladder distension. Distension of the bladder also produced a rise in blood pressure in some experiments; this effect has been reported previously by Mukherjee (1957*a*, *b*).

Reflex firing on sympathetic fibres in response to stimulation of vesical afferents. Electrical stimulation of the central end of the pelvic nerve elicited a reflex discharge on preganglionic nerves to the IMG and on ipsilateral and contralateral HGNs at latencies of 19–33 msec (mean 27 msec) and 45–100 msec (mean 60 msec), respectively (Figs. 3 and 6*D*). A response was recorded on various branches of the HGN (see Langley & Anderson, 1896 or Kuntz, 1929 for discussion of the anatomy): branches

to the ureter, to the pelvic plexus and a branch passing deep under the bladder, possibly providing an innervation to the rectum. The reflexes were present in animals with an intact spinal cord and in acute and chronic spinal animals.

In approximately 10% of the experiments, pelvic stimulation also elicited a discharge on the HGN at 150–350 msec latency. The late reflex occurred only at high intensities of stimulation (6–30 V) and persisted after transection of spinal cord (one experiment). When reflex responses were recorded from single preganglionic fibres, the units fired only a single action potential to a single stimulus to the pelvic nerve (Figs. 2*A*, *C*; 4*A*, *B*). The reflex latency for individual units ranged from 19 to 67 msec. The reflex discharge occurred consistently and failed only when preceded at close intervals by a spontaneously occurring action potential (Figs. 2*C*; 4*A*, *B*).

The threshold stimulus necessary to evoke a sympathetic discharge ranged in different experiments from 0·2 to 4 V, 0·05 msec duration (mean 1·1 V), whereas stimuli eliciting a maximal response varied from 1 to 10 V (mean 5 V, 3–8 times threshold) (Fig. 7). This range of voltages activated most of the myelinated afferent fibres in the pelvic nerve, but did not excite unmyelinated fibres.

The conduction velocities of pelvic afferent fibres evoking a sympathetic reflex discharge were measured in four preparations. Fig. 7*A* illustrates the results from one experiment. The reflex responses recorded on a hypogastric nerve to each of four stimulus intensities are shown in the right column (Fig. 7*A*, *b*) and the corresponding pelvic nerve action potentials at each intensity are shown in the left column (Fig. 7*A*, *a*). The most rapidly conducting afferents (30–45 m/sec) did not elicit a detectable response, but when slower conducting fibres were activated a large reflex was produced. The reflex reached a maximum amplitude at 3–4 times threshold (Fig. 7*B*). From the results of several experiments, it is concluded that the conduction velocity of the reflexogenic afferents is 6–25 m/sec.

Stimulating the pelvic nerve also evoked reflex volleys in the colonic nerves in five of sixteen experiments, but these responses were commonly of much lower amplitude than the HGN reflexes and occurred only at higher intensities (5–15 V) of stimulation. The latencies (50–100 msec) for colonic reflexes were comparable to those for HGN reflexes. Pelvic nerve stimulation did not evoke a reflex discharge on the cervical sympathetic trunk or on the post-ganglionic nerves from the superior cervical ganglion (four experiments).

The pelvic-lumbar sympathetic reflex was elicited only at low frequencies of stimulation. The responses were of maximal amplitude at

frequencies of one stimulus every 2–3 sec or longer, but were markedly reduced in amplitude at frequencies above 2 c/s (Fig. 8*B*). This characteristic of the reflex was also reflected in the long recovery period demonstrated by double shock stimulation. A single conditioning stimulus to the pelvic nerve was followed at various intervals by an identical stimulus to the same nerve. The time for complete recovery of the reflex varied considerably in different experiments, ranging from 0·4 to 1·4 sec (mean 0·8 sec). Fig. 8*D* illustrates the results of one experiment where the recovery

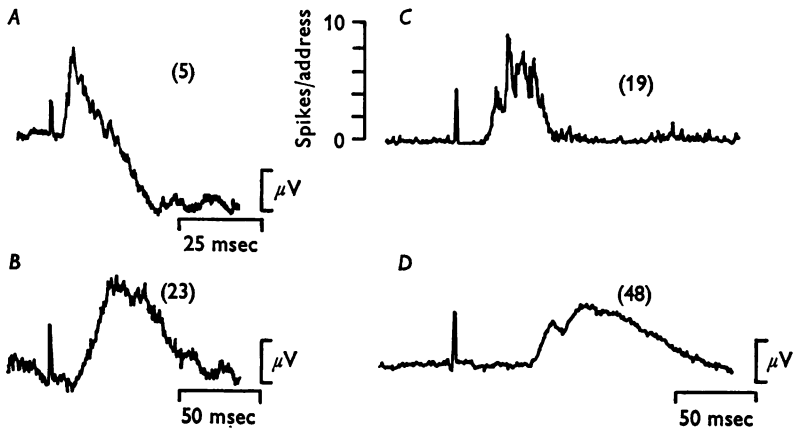

Fig. 6. Responses to pelvic nerve stimulation recorded at different points along the pelvic to lumbar sympathetic reflex pathway. Responses in records *A*, *B*, and *D* represent the average of twenty individual responses. Response in record *C* represents the total number of preganglionic spikes deposited in each address (0·25 msec/address) over eighty trials. Value in parentheses accompanying each record is the latency (msec) to the onset of the evoked response. *A*, cord dorsum potential recorded at S 2. *B*, cord dorsum potential recorded at L 4. *C*, reflex response recorded on ipsilateral lumbar preganglionic nerve. *D*, reflex response recorded on ipsilateral hypogastric nerve. Vertical calibrations: *A*, 60 μV; *B* and *D*, 35 μV, negativity upward. Time calibration in *D* also applies to *C*. Cat was anaesthetized with chloralose.

period was determined for the reflex discharge on both the HGN and a preganglionic nerve. This data shows clearly that the recovery cycle represents a property of the central pathway rather than the peripheral ganglionic pathway, which exhibited a shorter recovery cycle of 300–350 msec (see also Lloyd, 1939).

The recovery period for individual preganglionic fibres was much shorter than values obtained with multifibre preparations (Fig. 8*C*). Two units responded to a second volley at intervals of 120–150 msec, although the reflex latency of the second discharge was considerably prolonged at these intervals (Fig. 8*C*, *f–h*). Similarly, the occurrence of a spontaneous action

potential 10–150 msec before a reflexly evoked spike also could block the latter (Figs. 2*C* and 4*A*, *B*).

The central latency for the vesico-sympathetic reflex. This was determined by subtracting the conduction times for the peripheral afferent and efferent pathways from the total latency. Afferent fibres with conduction velocities between 6 and 25 m/sec were involved in the initiation of the sympathetic reflex. Peripheral afferent delay might vary, therefore, from

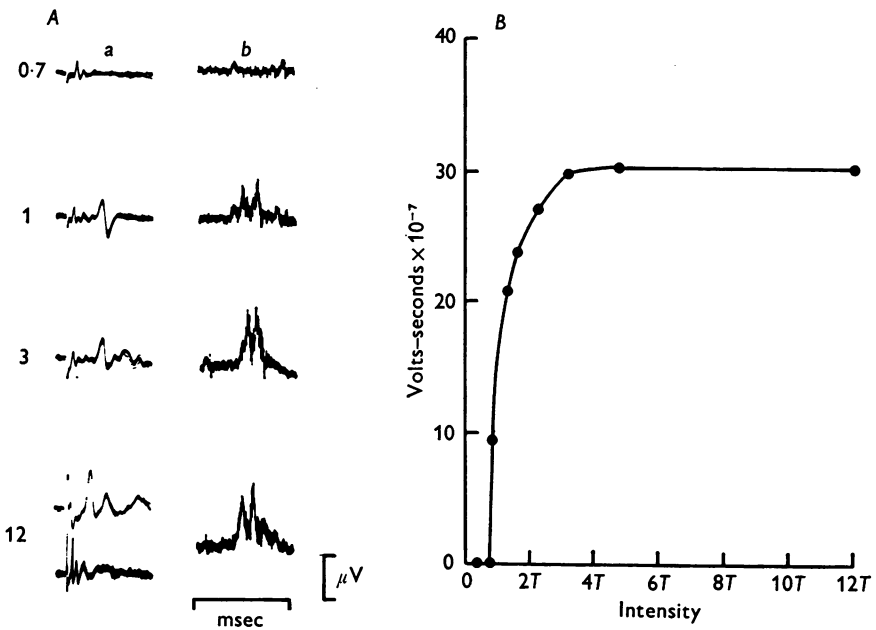

Fig. 7. *A*, relationship between pelvic nerve volleys (*a*) and hypogastric reflex responses (*b*) in a cat anaesthetized with chloralose. Stimuli applied to the pelvic nerve at 0·5 c/s; numerical values on the left margin denote stimulus intensity as multiples of the threshold for evoking a reflex. *B*, magnitude of reflex responses plotted against intensity of the stimulus delivered to pelvic nerve. Ordinate: area under averaged evoked potential expressed as volts-sec × 10^{-7}. Abscissa: intensity of stimulation expressed as multiples of reflex threshold (*T*). Vertical calibration in *A* represents 200 μV in *a* and 100 μV in *b*, negativity upward. Horizontal calibration represents 5 msec in *a*, except for the lower tracing where it represents 20 msec, and 200 msec in *b*.

3·5 to 16 msec for the peripheral conduction distance of 90–100 mm and, indeed, this figure is consistent with the observed latency (5–10 msec) for the sacral cord dorsum response elicited by stimulation of the central end of the pelvic nerve (Fig. 6*A*).

Efferent conduction times were estimated from the latencies for discharges on the HGN and preganglionic nerves to stimulation of the L2–

L4 ventral roots. Calculated preganglionic conduction velocities ranged from 6 to 15 m/sec, post-ganglionic velocities from 0·75 to 1·8 m/sec; and total conduction time to preganglionic and post-ganglionic recording sites was 5 and 30 msec, respectively. Utilizing these figures, estimated central latencies for the reflex varied in different experiments from 11 to 25 msec.

Central and peripheral pathways mediating the pelvic to sympathetic reflexes. The anatomy of the IMG of the cat and its preganglionic inner-

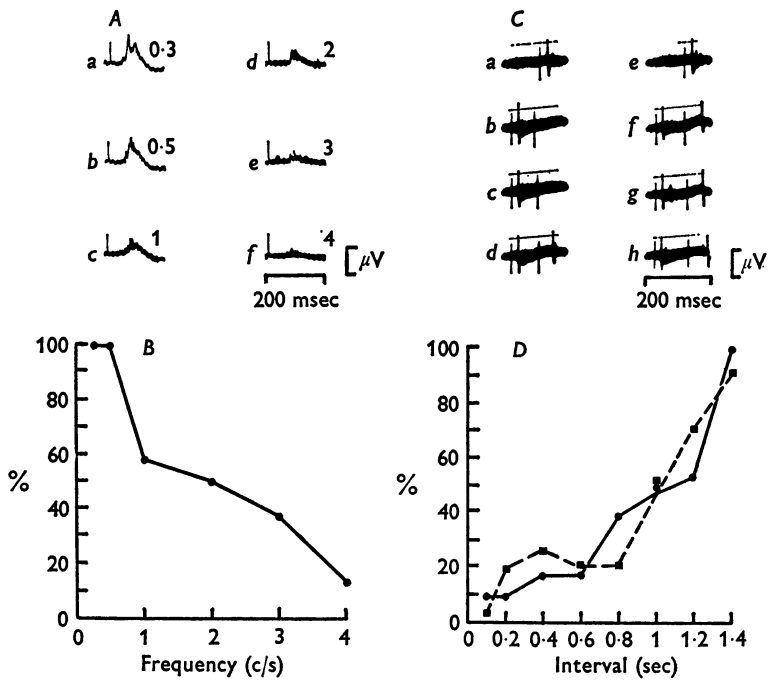

Fig. 8. *A* and *B* effect of frequency of stimulation on amplitude of pelvic to hypogastric reflex in two different experiments. Cats were anaesthetized with chloralose. *A*, reflex recorded on hypogastric nerve to supramaximal stimulation of pelvic afferent fibres at various frequencies. Numerical value above each record in *a–f* represents the frequency of stimulation in c/s. *B*, amplitude of pelvic to hypogastric reflex plotted against frequency of stimulation. Ordinate: amplitude as percent of maximum response. Abscissa: frequency of stimulation in c/s. *C* and *D*, time course of the depression of the pelvic to sympathetic reflex by a conditioning stimulus delivered to the same pelvic nerve. *C*, response of a preganglionic unit. Control responses, *a* and *e*; and responses evoked by conditioning and test stimuli at various intervals, *b–d*, and *f–h*. *D*, magnitude of the pelvic to preganglionic (■---■) and pelvic to hypogastric (●—●) reflexes plotted against the interval between conditioning and test stimuli. Ordinate: magnitude of test response expressed as % control. Abscissa: intervals between conditioning and test stimuli in sec.

vation has been described in detail (Langley & Anderson, 1896; Kuru, 1965). The IMG of each side receives from the rostral lumbar segments (L2–L5) preganglionic fibres which pass to the sympathetic chain and then to the IMG via the inferior splanchnic nerves. The latter are usually three or four in number and leave the chain near the 4th to 6th lumbar ganglia. The IMG of the two sides are connected by the interganglionic commissures lying above and below the inferior mesenteric artery.

The pelvic-HGN reflex must have a peripheral synapse in the IMG, since the reflex firing on the HGN was blocked by ganglionic blocking agents (e.g. tetraethylammonium or gamma aminobutyric acid) administered I.V. or by retrograde injection into the inferior mesenteric artery. On the other hand, firing in preganglionic fibres to the IMG was not depressed by the blocking agents, indicating that the pathway was not interrupted by synapses in the sympathetic chain ganglia.

It is known (Langley & Anderson, 1894; Lloyd, 1937) that preganglionic fibres arising on one side of the spinal cord make synaptic connexions in the contralateral as well as the ipsilateral IMG. The contralateral connexions are apparently made by fibres crossing in the interganglionic commissures. Experiments were conducted to determine whether the sympathetic reflexes evoked by pelvic nerve stimulation followed both the crossed and uncrossed pathways. Since sectioning of all preganglionic nerves to the left IMG did not abolish the reflex in the left HGN (Fig. 9*C*), but a subsequent transection of all preganglionic nerves to the right (contralateral) IMG did abolish the discharge, it is concluded that the reflex pathway is partially crossed at the ganglion level.

In the spinal cord the reflex pathway is also partially crossed. Transection of the dorsolateral quadrant of the cord on one side between L7 and L4 commonly abolished or markedly reduced the pelvic-evoked firing in an ipsilateral preganglionic nerve or HGN, but at the same time enhanced or did not change the responses in contralateral nerves (Fig. 9). Since sections at any point between L7 and L4 were equally effective, the site of crossing must be primarily at the sacral level. The results were the same regardless of whether pelvic nerve stimulation was ipsilateral or contralateral to the recording site. In some experiments, the reflex in the HGN ipsilateral to the section was not abolished completely due to crossing of preganglionic fibres at the ganglion level (*vide supra*).

Effect of bladder pressure on the pelvic to sympathetic reflex. Spontaneous or induced changes in bladder pressure altered the amplitude of the pelvic to sympathetic reflex. In animals with an intact spinal cord, large bladder contractions occurring at constant volume were invariably associated with a marked or complete suppression of the reflex recorded on either preganglionic (Fig. 3*B*) or HGNs (twenty-one experiments, Fig. 10 and 11).

The depression occurred with the onset of pelvic efferent firing and either during the rising phase or often slightly before the commencement of a bladder contraction (Fig. 10*E*). The reflex recovered on the declining phase of the bladder contraction (Fig. 10*E*). When bladder contractions exhibited an irregular peak, with lower amplitude, transient contractions superimposed upon a larger more sustained contraction, it was often observed that the reflex recovered during the falling phase of these small contractions and was inhibited during the rising phase, even though mean intra-

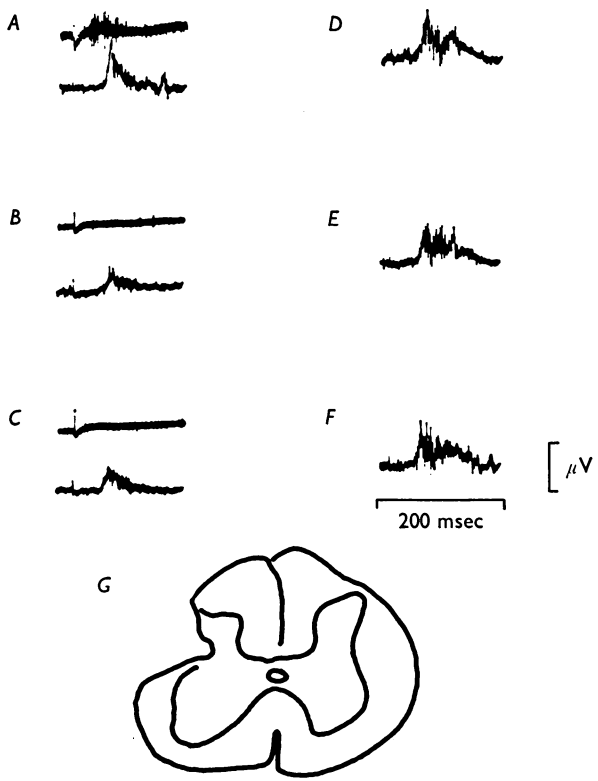

Fig. 9. Sympathetic reflexes elicited by pelvic nerve stimulation before and after partial section of the spinal cord in a cat under chloralose anaesthesia. Reflexes recorded on nerves ipsilateral (*A*, *B*, *C*, left side) and contralateral (*D*, *E*, *F*, right side) to site of stimulation (left pelvic nerve). Upper traces in *A*, *B*, and *C* are preganglionic responses; lower traces are responses recorded on hypogastric nerves. Records in *D*, *E*, and *F* are responses on contralateral hypogastric nerve. *A* and *D*, control responses. *B* and *E*, responses recorded 2 min after a small incision in left dorsolateral quadrant between L 4 and L 5. *C* and *F*, responses recorded after cutting all preganglionic nerves to left inferior mesenteric ganglia. The extent of the lesion is depicted in *G*. Vertical calibration in *A*–*F* is 100 μV, negativity upward.

vesical pressure was at a high level throughout (Fig. 10*E*). Thus, it would appear that inhibition is not related primarily to intravesical pressure, but rather to the presence of an active bladder contraction, mediated by firing in the excitatory neural pathway to the bladder (i.e. the sacral parasympathetic outflow). At the same time that the pelvic-HGN reflex was

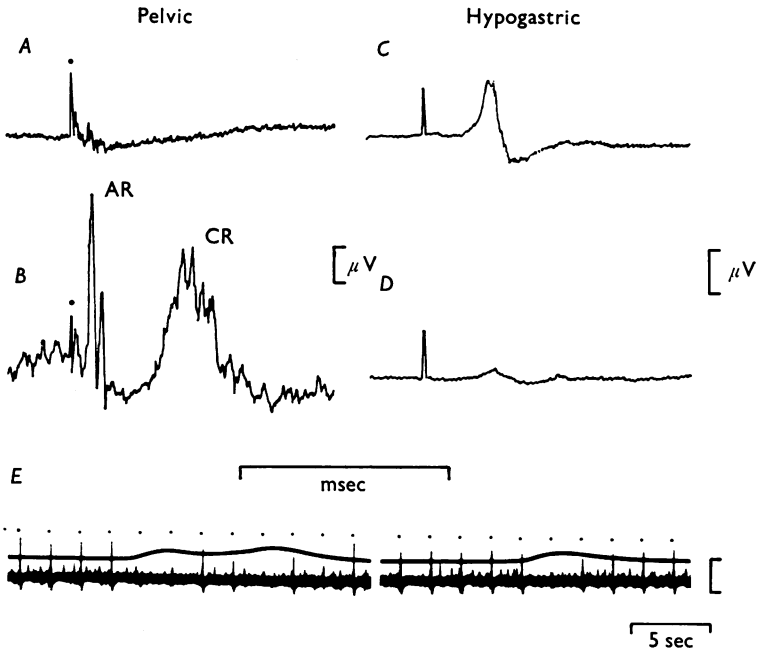

Fig. 10. Relationships between bladder activity and reflexes evoked in pelvic (*A*, *B*) and hypogastric nerves (*C*, *D*, *E*) by electrical stimulation of the pelvic nerve. Cat was anaesthetized with chloralose. Records *A–D* are computer-averaged responses recorded between (*A* and *C*) and during (*B* and *D*) isovolumetric bladder contractions. Each record is the average of ten evoked responses. *A* and *B*, efferent responses recorded on a pelvic nerve filament. In record *B*, AR and CR refer to axon reflex and central reflex responses, respectively (de Groat & Ryall, 1969). *C* and *D*, central reflex responses recorded on the peripheral end of a hypogastric nerve. *E*, continuous film records of bladder pressure (upper trace) and reflex responses (lower trace) on the same hypogastric nerve as in *C* and *D*. Single volleys were delivered to the central end of a pelvic nerve at a rate of 0·5 c/s as indicated by dots. Vertical calibration in record *E* is 100 cm H_2O (upper trace) and 40 μV (lower trace).

depressed the post-ganglionic action potentials elicited on the HGN by preganglionic nerve stimulation were either increased in amplitude or unchanged and reflexes on the pelvic nerve were enhanced (Fig. 10*B*).

Inhibition was also elicited by artificially inducing a micturition reflex by injecting fluid into the bladder under constant volume conditions or by

maintaining the bladder at constant pressure above the threshold (10–20 cm H_2O) for inducing a micturition contraction (thirteen experiments, Fig. 11*B*). In a few experiments intravesical pressures below the micturition threshold (5–10 cm H_2O) facilitated the pelvic-HGN reflex. Other reflexes to the lumbar sympathetic outflow were not affected by bladder activity in either normal or spinal animals. Stimulation of the central end of a preganglionic nerve to the IMG (lumbar visceral afferents) or stimulation of the carotid sinus nerve (W. C. de Groat & P. M. Lalley, unpublished observations) evoked reflex firing on post-ganglionic sympathetic

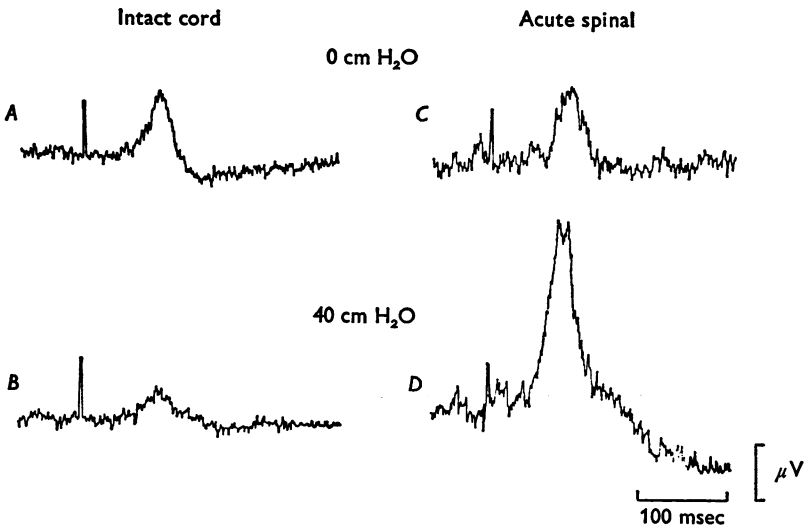

Fig. 11. Effect of bladder distension on the pelvic to hypogastric reflex before (*A* and *B*) and 2 min after (*C* and *D*) transecting the spinal cord at T11. Records represent the average of ten individual responses. *A*, reflex response on the ipsilateral hypogastric nerve to stimulation of pelvic afferents (0·5 c/s). Bladder pressure held constant at 0 cm H_2O. *B*, inhibition of reflex response at 40 cm H_2O bladder pressure. *C*, reflex response at 0 cm H_2O pressure after spinal transection. *D*, enhancement of the reflex at 40 cm H_2O pressure in spinal animal. Vertical calibration 40 μV, negativity upward. Cat was anaesthetized with chloralose.

nerves (HGN and colonic) at average latencies of 50 and 120 msec, respectively. These reflexes were neither enhanced nor depressed by alterations in bladder pressure. Similarly, the reflex discharge evoked on the colonic nerve by pelvic nerve stimulation was also unaffected by an increase in intravesical pressure in five of six experiments. Activation of carotid baroreceptors by distension of the carotid sinus or I.V. injection of noradrenaline (2–6 μg/kg) did not depress the pelvic-sympathetic reflex.

Since it was shown in a previous study (de Groat & Ryall, 1969) that

excitatory reflexes to the bladder were mediated by a supraspinal pathway, it was of interest to study the effect of spinal transection on the depression of the pelvic-HGN reflex. Following acute transection of the cord at T10 to T12, micturition reflexes were absent and inhibition of the pelvic-HGN reflex was no longer obtained by raising bladder pressure even to 60 cm H_2O. Instead, the reflex was enhanced, starting at a threshold of 8–10 cm H_2O and reaching a maximal enhancement at 25–40 cm H_2O (Fig. 11*D*). The reflex was also enhanced during the low amplitude contractions of the bladder which persisted after spinal transection. These contractions reflect the intrinsic activity of the vesical smooth muscle and are unrelated to central neuronal mechanisms (Plum, 1960; de Groat & Ryall, 1969). In non-spinal animals the bladder-induced inhibition of the pelvic-HGN reflex was also abolished when the animal's condition deteriorated during lengthy experiments. When systolic blood pressure dropped below 75–100 mm Hg the micturition reflex was absent; raising bladder pressure then failed to block the sympathetic reflex. In addition, in a few experiments in which the animal was in poor condition at the start (i.e. low blood pressure, absence of pupillary reflex), the micturition reflex and inhibition were also unobtainable.

The absence of inhibition following transection of the spinal cord would suggest that the inhibition was mediated either by a supraspinal pathway, or by a spinal pathway dependent upon a bulbospinal facilitation. In an attempt to differentiate between these two possibilities, experiments were conducted in chronic spinal animals to determine whether the inhibition reappeared after the animal had recovered from 'spinal shock'. In two of four chronic spinal animals in which automatic micturition had developed (de Groat & Ryall, 1969), a depression of pelvic to sympathetic reflexes was again observed during isovolumetric bladder contractions. However, in contrast to the findings in normal animals, the reflex was not depressed in any of the chronic spinal animals when bladder pressure was raised artificially; on the contrary the reflex was enhanced, as found in the acute spinal preparations. In chronic spinal animals two preganglionic units were also studied. These units were activated by pelvic nerve stimulation and fired during a bladder contraction. The firing elicited by pelvic stimulation was not depressed by raising bladder pressure.

DISCUSSION

It was shown in the present experiments that activation of vesical afferent fibres by electrical stimulation or by bladder distension elicited reflex responses in the lumbar sympathetic outflow. The afferent fibres ($A\gamma\delta$) which produced the reflex apparently arose from tension or stretch

receptors in the bladder wall and reached the sacral cord via the pelvic nerves. The presence of the reflex after spinal transection (T 10–T 12) and the long central latency (mean 18 msec) denote a multisynaptic spinal reflex.

The salient features of the vesico-sympathetic reflex differ in a number of respects from sympathetic responses evoked by other afferent fibres. The resistance of the reflex to spinal shock is especially noteworthy. Both Sato & Schmidt (1971) and Coote & Downman (1966) observed initial complete block after cervical cord transections of spinal sympathetic reflexes evoked by stimulation of spinal sensory nerves. In the present study, reflex depression was never observed, even at the earliest intervals following transection. It is also noteworthy that pelvic afferents did not produce a late supraspinal discharge in the lumbar sympathetic outflow, whereas in other studies (Coote & Downman, 1966; Coote, Downman & Weber, 1969; Sato & Schmidt, 1971; Sato *et al.* 1969), supraspinal reflexes evoked by stimulation of visceral or somatic afferents were more prominent than spinal reflex responses. The estimated central latency for the vesico-sympathetic reflex was somewhat longer than similar estimates for other spinal sympathetic reflexes (Beacham & Perl, 1964*a*, *b*; Franz, Evans & Perl, 1966; Coote & Downman, 1966; Sato & Schmidt, 1971); however, this longer central delay may reflect the intersegmental distribution of the pathway.

The sympathetic discharges elicited by moderate increases in bladder pressure or by electrical stimulation of the pelvic nerves were unaffected by carotid sinus distension or by pressor doses of noradrenaline. This would indicate that these vesico-sympathetic reflexes are resistant to baroreceptor inhibition. On the other hand, sympathetic reflexes elicited by extreme bladder distension, where the afferent limb lies in the HGN, (Talaat, 1937) are reportedly depressed by baroreceptor activation (Mukherjee, 1957*a*, *b*).

Like other autonomic reflexes, the vesico-sympathetic reflex was depressed by increasing rates of stimulation, optimal frequencies being less than 1 stimulus per second. The frequency dependence was also reflected in the prolonged recovery periods (0·4–1·4 sec) demonstrated with the double shock technique. Prolonged recovery of autonomic reflexes has been linked by other investigators (Coote & Perez-Gonzalez, 1970) to supraspinal reflex mechanisms. It is now evident that this phenomenon can also be a feature of spinal reflex pathways.

In animals with an intact spinal cord, depression of the electrically evoked sympathetic reflex was observed during bladder distension or spontaneous bladder contractions. Since sympathetic firing increased at elevated bladder pressures, it is possible to account for the depression of

the reflex by at least two different mechanisms: (1) refractoriness in the spinal pathway as a result of the increased sympathetic firing, or (2) active inhibition. It is concluded, however, that inhibition is primarily responsible for the depression since: (1) spinal transection (T 10–T 12) abolished the depression elicited by bladder distension without diminishing the asynchronous discharge to raised bladder pressure, (2) in chronic spinal cats bladder distension produced intense sympathetic firing but no depression of the electrically evoked responses and (3) reflex depression could also occur when bladder contractions were accompanied by a decrease in spontaneous sympathetic firing (Fig. 4). The absence of inhibition in spinal animals also indicates that the inhibitory process must be mediated or at least facilitated by supraspinal mechanisms.

The major question to be answered then is 'what role do the inhibitory and excitatory vesico-sympathetic reflexes play in the regulation of bladder function?' It is known that the sympathetic innervation to the bladder of the cat provides an inhibitory input to the vesical parasympathetic ganglia (de Groat & Saum, 1971*a*, *b*, *c*) and an excitatory and inhibitory input to the vesical smooth muscle (Kuru, 1965; Edvardsen, 1968 *a*, *b*, *c*; de Groat & Saum, 1971*c*). Edvardsen (1968*a*, *b*, *c*) has suggested on the basis of cystometrographic data that bladder distension activates a sympathetic inhibitory pathway to the bladder. He presented evidence that the pathway was organized at the spinal level, which is consistent with the present results, and that it was activated during bladder filling. He showed, as did Gjone (1965) and Langworthy *et al.* (1940) that section of the hypogastric nerves led to increased bladder activity and a shift to the left in bladder pressure–volume curves (cystometrograms). Accordingly, he concluded that the sympathetic reflex might be one of several mechanisms involved in the maintenance of urinary continence.

In support of Edvardsen's proposals, we have observed increased sympathetic firing at bladder pressures below the micturition threshold; however, we have also recorded intense sympathetic firing during micturition, as well. This is somewhat unexpected in view of the proposed vesical inhibitory role of the sympathetic nerves; since one might anticipate the opposite response, i.e. a decrease in sympathetic activity during micturition, thereby facilitating bladder emptying. On the other hand, a depression of the sympathetic reflex evoked by pelvic nerve stimulation was observed during bladder contractions and preganglionic units were encountered which were fired by pelvic stimulation, but which were depressed during micturition. Thus, it appears that there are at least two distinct populations of lumbar preganglionic fibres which are involved in vesico-sympathetic reflexes: one population which is activated and another which is depressed during the micturition reflex. We might expect in accord with

the conclusions of Edvardsen (1968*a*, *b*, *c*) that the latter population participates in an inhibitory feed-back mechanism on to the bladder. This population seems to be inhibited by a supraspinal mechanism during micturition. The second population of preganglionic fibres is apparently unaffected by this supraspinal inhibition, and might be expected to subserve other visceral regulatory functions.

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REFERENCES

BEACHAM, W. S. & PERL, E. R. (1964*a*). Background and reflex discharge of sympathetic preganglionic neurones in the spinal cat. *J. Physiol.* **172**, 400–416.

BEACHAM, W. S. & PERL, E. R. (1964*b*). Characteristics of a spinal sympathetic reflex. *J. Physiol.* **173**, 431–448.

BRADLEY, W. E. & TEAGUE, C. T. (1969). Hypogastric and pelvic nerve activity during the micturition reflex. *J. Urol.* **102**, 438–440.

COOTE, J. H. & DOWNMAN, C. B. B. (1966). Central pathways of some autonomic reflex discharges. *J. Physiol.* **183**, 714–729.

COOTE, J. H., DOWNMAN, C. B. B. & WEBER, W. V. (1969). Reflex discharges into thoracic white rami elicited by somatic and visceral afferent excitation. *J. Physiol.* **202**, 147–159.

COOTE, J. H. & PEREZ-GONZALEZ, J. F. (1970). The response of some sympathetic neurones to volleys in various afferent nerves. *J. Physiol.* **208**, 261–278.

DE GROAT, W. C. & RYALL, R. W. (1968). Recurrent inhibition in sacral parasympathetic pathways to the bladder. *J. Physiol.* **196**, 579–591.

DE GROAT, W. C. & RYALL, R. W. (1969). Reflexes to sacral parasympathetic neurones concerned with micturition in the cat. *J. Physiol.* **200**, 87–108.

DE GROAT, W. C. & SAUM, W. R. (1971*a*). Sympathetic-inhibition of the urinary bladder and of pelvic parasympathetic ganglionic transmission in the cat. *Fedn Proc.* **30**, 656.

DE GROAT, W. C. & SAUM, W. R. (1971*b*). Adrenergic inhibition in mammalian parasympathetic ganglia. *Nature, New Biol.* **231**, 188–189.

DE GROAT, W. C. & SAUM, W. R. (1971*c*). Sympathetic-inhibition of the urinary bladder and of pelvic ganglionic transmission in the cat. *J. Physiol.* **214**, 297–314.

EDVARDSEN, P. (1968*a*). Nervous control of urinary bladder in cats. I. The collecting phase. *Acta physiol. scand.* **72**, 157–171.

EDVARDSEN, P. (1968*b*). Nervous control of urinary bladder in cats. II. The expulsion phase. *Acta physiol. scand.* **72**, 172–182.

EDVARDSEN, P. (1968*c*). Nervous control of urinary bladder in cats. III. Effects of autonomic blocking agents in the intact animal. *Acta physiol. scand.* **72**, 183–193.

FRANZ, D. N., EVANS, M. H. & PERL, E. R. (1966). Characteristics of viscero-sympathetic reflexes in the spinal cat. *Am. J. Physiol.* **211**, 1292–1298.

GJONE, R. (1965). Peripheral autonomic influence on the motility of the urinary bladder in the cat. I. Rhythmic contractions. *Acta physiol. scand.* **65**, 370–377.

KUNTZ, A. (1929). In *The Autonomic Nervous System*, 1st edn., pp. 277–299. Philadelphia: Lea and Febiger.

KURU, M. (1965). Nervous control of micturition. *Physiol. Rev.* **45**, 425–494.

LALLEY, P. M., DE GROAT, W. C. & MCLAIN, P. L. (1971). Reflex firing on the hypogastric nerve (HGN) in response to stimulation of vesical afferent fibers. *Fedn Proc.* **30**, 324.

LANGLEY, J. N. & ANDERSON, H. K. (1894). On reflex action from sympathetic ganglia. *J. Physiol.* **16**, 410–440.

LANGLEY, J. N. & ANDERSON, H. K. (1895). The innervation of the pelvic and adjoining viscera. Part II. The bladder. *J. Physiol.* **19**, 17–84.

LANGLEY, J. N. & ANDERSON, H. K. (1896). The innervation of the pelvic and adjoining viscera. Part VII. Anatomical observations. *J. Physiol.* **20**, 372–406.

LANGWORTHY, O. R., KOLB, L. C. & LEWIS, L. G. (1940). *Physiology of Micturition.* Baltimore: Williams and Wilkins Company.

LLOYD, D. P. C. (1937). The transmission of impulses through the inferior mesenteric ganglia. *J. Physiol.* **91**, 296–313.

LLOYD, D. P. C. (1939) The excitability states of inferior mesenteric ganglion cells following preganglionic activation. *J. Physiol.* **95**, 464–475.

MUKHERJEE, S. R. (1957*a*). Effect of bladder distension on arterial blood pressure and renal circulation in acute spinal cats. *J. Physiol.* **138**, 300–306.

MUKHERJEE, S. R. (1957*b*). Effect of bladder distension on arterial blood pressure and renal circulation: role of splanchnic and buffer nerves. *J. Physiol.* **138**, 307–325.

PLUM, F. (1960). The genesis of vesical rhythmicity. *Archs Neurol. Psychiat., Chicago* **2**, 487–496.

SATO, A., KAUFMAN, A., KOIZUMI, K. & MCBROOKS, C. (1969). Afferent nerve groups and sympathetic reflex pathways. *Brain Res.* **14**, 575–587.

SATO, A. & SCHMIDT, R. F. (1971). Spinal and supraspinal components of the reflex discharges into lumbar and thoracic white rami. *J. Physiol.* **212**, 839–850.

TALAAT, M. (1937). Afferent impulses in the nerves supplying the urinary bladder. *J. Physiol.* **89**, 1–13.