13. Hunt, W. G., Moore, R. O., *Applied Microbiol.*, 1958, v6, 36.

14. Maschmann, E., *Biochem. Z.*, 1937, v294, 1.

15. Weil, L., Kocholaty, W., Smith, L. D., *Biochem. J.*, 1939, v33, 893.

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Spontaneous Activity in Gamma Efferents of a Deafferented Spinal Cord Segment. (25540)

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Isolation of spinal cord segment from descending and afferent impulses leads in puppies(1), cats(2) and monkeys(3) to contractile inactivity of parts innervated by the segment. Even the spinal dogfish, which has incessant spontaneous swimming movements when dorsal roots are intact, becomes quiet when sufficient roots are cut(4). Absence of overt contractions, of course, indicates that there is no firing of the large alpha motoneurons which innervate extrafusal muscle fibers. Not eliminated, however, is the possibility of unrevealed activity in the small fusimotor neurons or gamma efferents leading to intrafusal fibers of muscle spindles. Under many conditions of physiological and experimental stimulation, spindle loops may be in vigorous fluctuating activity without alpha motoneuron participation(5). The firing of these fusimotor neurons can be influenced from both segmental and supraspinal levels(6). Bilateral exclusion of dorsal root inflow does not extinguish unevoked activity in these neurons in animals with intact supraspinal connections(5); nor does interruption of descending pathways prevent continuing activity if segmental inflow is intact(7). In our experiments, both segmental afferent and descending influences have been excluded from the cat's lumbosacral cord to see if activity in fusimotor fibers may be fully independent. Evidence of spontaneous activity of fusimotor neurons under these conditions has been obtained in acute and chronic preparations.

Methods. The lumbosacral segment of spinal cord in 16 cats was isolated from impinging nervous impulses by transecting at L_1 and cutting all dorsal roots to the segment. Transection in acute experiments was preceded by freezing of the cord at immediate site of sectioning; in chronic preparations, either the same method was used, or dura was left intact and a cut made between ligatures tied around the dural sac. Dorsal roots were clamped with silver clips and cut extradurally if deafferentation was performed at time of initial operation, or if left to terminal experiment, the roots were cut along line of their entrance into the cord, at which level it was generally possible to spare major radicular vessels. When cutting extradurally, ventral roots from about S_3 caudalward also had to be sacrificed. Close nursing care involving treatment with antibiotics, forced feeding, and bladder expression was given to chronic animals over 2-30 days. At time of terminal observation, cats were electrolytically decerebrated, the lumbosacral cord bathed in mineral oil maintained within 1° of normal body temperature by thermistor-controlled radiant heat, and observations begun 2-6 hours after surgery was completed. Potentials were detected by suspending fine filaments of either ventral or dorsal roots on submerged silver electrodes. When dorsal root fibers were monitored, the hind leg was denervated except for the nerve to triceps surae, and units were identified as spindle afferents by presence of a pause in discharge during induced twitch. Checks were routinely made for completeness of deafferentation by inspection for reflex responses upon stimulation of the skin, through search for afferent activity in dorsal roots in preparations with extradurally cut roots, and by postmortem inspection of root and cord lesions.

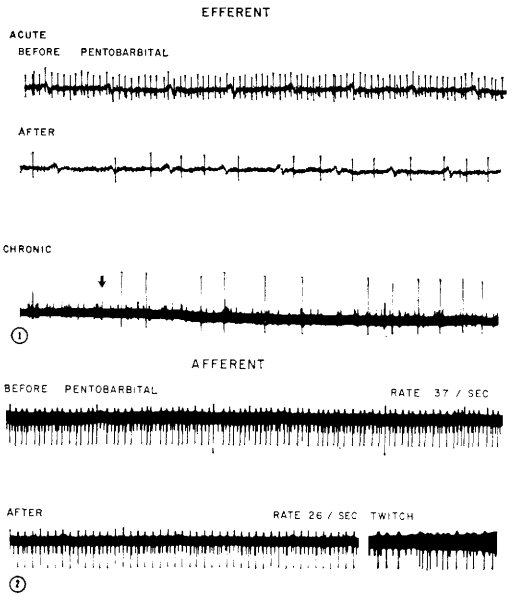

FIG. 1. A. Efferent unit from S_1 VR of cat in which cord was transected at L_2 and lumbosacral segment deafferented 5 hr previously. Midbrain decerebration. Discharge is shown before and after slowing of the unit by administration of intrav. pentobarbital. This unit had been monitored for one hr before the upper trace was taken. B. Efferent spikes in S_1 VR filament 8 days after transection and deafferentation. Arrow indicates that dorsum of cord was touched to excite large alpha spikes seen contrasting with the background of gamma efferent discharge.

FIG. 2. Unit in S_1 DR of acute preparation before and after pentobarbital. Discharge dropped from 37/sec. to 26/sec. and became more regular. Twitch response at lower right identifies unit as a spindle afferent in the triceps surae muscle. Muscle tension remained unchanged.

Results. Presence of activity in ventral roots. Total absence of spontaneous phasic or tonic contractions and marked degree of atrophy in those muscles innervated by the chronic deafferented spinal cord segment of these adult cats accords with similar observations from cats(2), puppies(1), and monkeys (3). Only when pressure was applied along the mid-dorsal scar would movements of hind limbs occur, probably as a result of direct irritation of the cord(1). This did demonstrate that the cord was viable, and that alpha motoneurons could be aroused to fire. Despite absence upon simple observation of spontaneous movements in hindquarters of chronic deafferented spinal cats, these animals after surgical preparation for recording, demonstrated sustained spiking discharge in ventral roots of the isolated cord segment (Fig. 1, lowermost trace). Single units, firing at rates of 6-25/sec., and with fair regularity, could be followed for over a half hour. Administration of ether or intravenous injection of pentobarbital caused slowing and final silencing of this discharge.

In 2 animals, to lessen the chance for occurrence of an episode of cord ischemia, cutting of dorsal roots was delayed until terminal experiment 7 or 10 days later. Several acutely transected and deafferented preparations were also examined to avoid denervation sensitization (Fig. 1, upper traces). Ventral roots in both of these types of preparation showed a discharge similar to that of chronically deafferented spinal preparation.

Identification of activity. Spontaneously discharging spikes were small contrasted to other spikes which could be aroused to fire by electrical or mechanical stimulation of surface of cord (Fig. 1, lowermost trace). This, together with absence of overt contractile activity suggested that small spikes arose in fusimotor fibers leading to muscle spindles, and the large ones of temporary appearance in motoneurons innervating extrafusal muscle. Continuing action in fusimotor fibers to a spindle should 1) enhance the rate of afferent discharge from that spindle, and 2) induce irregularity in its discharge(5). Fig. 2 illustrates these effects in discharge from a spindle unit in the medial gastrocnemius muscle of a cat in which the cord and dorsal roots were severed 2 hours before observations. In the upper trace, the afferent is firing at a rate of 37/sec., and wavelike irregularity of the intervals is evident. The lower record, taken after intravenous administration of an anesthetic dose of pentobarbital, shows a changed picture in which the rate is 26/sec., and the intervals are regular. As pentobarbital has only minor direct effect on spindle afferents, these changes were due to elimination of tonic discharge in fusimotor fibers to that spindle.

Discussion. The question arises whether the activity detected in these preparations is an attribute of normal cord function or an artifact admitted by severe conditions of the

experiment. Temporary ischemia of the spinal cord, for example, might have led to loss of interneurons, decrease in net inhibition, and resultant sustained discharge of motoneurons including gamma units(8). However, in our chronic animals, ischemic rigidity was avoided, since arteries entering along ventral roots were undisturbed, those along dorsal roots were spared and, in some preparations, compensatory adjustments in circulation were favored by making the transection and deafferentation at different times. Lesser effects due to relative ischemia of long duration, or to sensitization of denervated neurons are excluded in animals prepared acutely.

The flurry of injury discharge which follows procedure of isolating a ventral root filament may dwindle to a slow discharge that must be distinguished from spontaneous activity, particularly since myelinated axons of small size are more prone to demonstrate injury potentials(9). That units were not accepted as truly spontaneous unless firing was sustained for over 5 minutes, and that some were followed for periods to an hour is reassuring. Moreover, injury potentials in ventral roots due to handling were eliminated as a factor in experiments in which afferent units were monitored. Triggering of cord activity from hyperexcitable tissue lying adjacent to cord transection is a further possibility.

The volume of discharge heard in ventral rootlets of these preparations is not as great as the similarly pure fusimotor activity in roots leading from deafferented levels of the cord in decerebrate cats. This and the observation that silent units of probable fusimotor nature can be aroused to activity with stimulation of the isolated cord segment suggests that only a minor portion of available gamma units is spontaneously active.

Summary. Presence of spiking discharge in fibers of ventral roots leading from lumbosacral cord completely deafferented and transected at the L_1 level is described. This activity was noted in adult cats either chronically or acutely prepared. On the bases of the small relative height of these spikes and the demonstration in afferent discharge from muscle spindles of tonic fusimotor influence, it is concluded that ventral root activity arises in gamma efferent fibers. Probably only a minor portion of gamma fibers present is participating.

1. Tower, S., *J. comp. Neurol.,* 1937, v67, 109.

2. Eccles, J. C., *J. Physiol.,* 1944, v103, 253.

3. Tower, S., Bodian, D., Howe, H., *J. Neurophysiol.,* 1941, v4, 388.

4. Lissmann, H. W., *J. Exp. Biol.,* 1946, v23, 162.

5. Eldred, E., Granit, R., Merton, P., *J. Physiol.,* 1953, v122, 498.

6. Granit, R., *Receptors and Sensory Perception,* Yale University Press, 1955.

7. Hunt, C. C., *J. Physiol.,* 1951, v115, 456.

8. Gelfan, S., Tarlov, I. M., *ibid.,* 1959, v146, 594.

9. Adrian, E. D., *Proc. Roy. Soc. B,* 1930, v106, 596.

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Experimental Shigella Infections. III. Sensitivity of Normal, Starved and Carbon Tetrachloride Treated Guinea Pigs to Endotoxin. (25541)

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Hartley strain guinea pigs may be rendered susceptible to a fatal enteric infection with a strain of *Shigella flexneri* 2a if they are either deprived of food for 4 days prior to challenge or injected subcutaneously with carbon tetrachloride 24 to 48 hours before oral administration of viable bacteria(1,2). The mechanism of this increased susceptibility is not clear.