16237

Early Effects of Denervation Upon Response of Muscle to Continuous Stimulation.

DWIGHT J. INGLE AND JAMES E. NEZAMIS.

From the Research Laboratories, The Upjohn Company, Kalamazoo, Mich.

The gastrocnemius muscle of the normal rat can sustain a high rate of work for several days in response to direct faradic stimulation.[1] The purpose of this study was to determine the early effects of cutting the sciatic nerve upon the ability of the gastrocnemius muscle to sustain its responsiveness. It was found that the response of the denervated muscle to a standard stimulus was decreased below that of sham-operated animals within 24 hours after the nerve was cut and continued to decrease rapidly until responsiveness was almost completely lost.

Methods. Male rats of the Sprague-Dawley strain were used. The control and experimental animals were closely matched into pairs according to weight which ranged from 310 to 350 g. The sciatic nerve was cut just above the level of the knee joint. In the control animals the nerve was exposed but not cut. The operations in Exp. 1 were done under barbiturate anesthesia, and the animals were subjected to the work test immediately following operation. The operations in Exp. 2 and 3 were done under ether anesthesia, since there was a delay of 24 to 48 hours in starting the work test. The animals were prepared for the work test by the subcutaneous injection of phenobarbital sodium and the intraperitoneal injection of cyclopal sodium. Anesthesia was maintained throughout the experiment by the repeated subcutaneous injection of phenobarbital sodium.

The animals were placed upon an animal board, enclosed in a temperature cabinet (28°C) and prepared for the stimulation of muscle to lift 100 g 3 times per second, according to the procedure of Ingle.[2] A silver needle placed in the biceps of the thigh served as one electrode, and a hemostat, which was clamped on the lower tibia, served as the second electrode. The muscular contractions were registered on automatic work adders. The relative amounts of work are expressed as recorded revolutions, and rates of work as number of recorded revolutions per minute (RPM). Each recorder revolution represents approximately 400 g-cm of work. All of the apparatus used was refined over that previously described[2] but the principles of method have remained the same. An electrical stimulator (Nerve Stimulator, Model B, Upjohn) was used which had adjustable amplitude, wave shape, pulse length and pulse frequency. A cathode ray oscilloscope was incorporated into the stimulator for observing and calibrating the output. A small amount of AC current was imposed on that of the DC pulse. The frequency of pulse was 3 per second, its duration was 20 milliseconds and its intensity was 12 milliamperes.

Experiments and Results. In Exp. 1, 12 rats having the sciatic cut and 12 rats having sham operations were subjected to direct stimulation of the muscle immediately following operation. The rates of work were recorded at the beginning of the experiment and 24, 30, 36, 48, 54, 60, and 72 hours later. The average rate of work for denervated muscle was depressed below that of the control animals at 24 hours and decreased rapidly thereafter so that only feeble responses were recorded after 48 hours. At the end of the 72-hour test period the intensity of the stimulus was increased from 12 to 20 milliamperes. The average rate of work increased from 0.5 to 13.2 RPM in the denervated muscles and from 10.2 to 15.7 RPM in the control series. The total number of recorder revolutions for the denervated series was 24,764 and for the control series 51,655.

[1] Heron, W. T., Hales, W. M., and Ingle, D. J., *Am. J. Physiol.*, 1934, **110**, 357.

[2] Ingle, D. J., *Endocrinology*, 1944, **34**, 191.

In Exp. 2, 12 rats were denervated under ether anesthesia, and 24 hours later they were anesthetized with the barbiturates and subjected to direct stimulation of the muscle. The initial level of work was subnormal and decreased rapidly so that muscular responsiveness was almost lost by 24 hours. When the intensity of stimulation was raised to 20 milliamperes the average rate of work increased from 0.1 to 18.5 RPM.

In Exp. 3, 12 rats were denervated and 12 rats were sham-operated under ether anesthesia, and 48 hours later they were anesthetized with the barbiturates and subjected to direct stimulation of the muscle for 24 hours. The initial rates of work were normal for the sham-operated rats and subnormal for the denervated series. Muscular responsiveness decreased rapidly in the latter series so that by 24 hours muscular contractions were very weak. When the intensity of stimulation was raised to 20 milliamperes the average rate of work was increased from 1.0 to 13.7 RPM in the denervated series and from 12.8 to 21.0 RPM in the control series. The total amount of work for the denervated series was 6,638 recorder revolutions and 22,187 recorder revolutions in the control series.

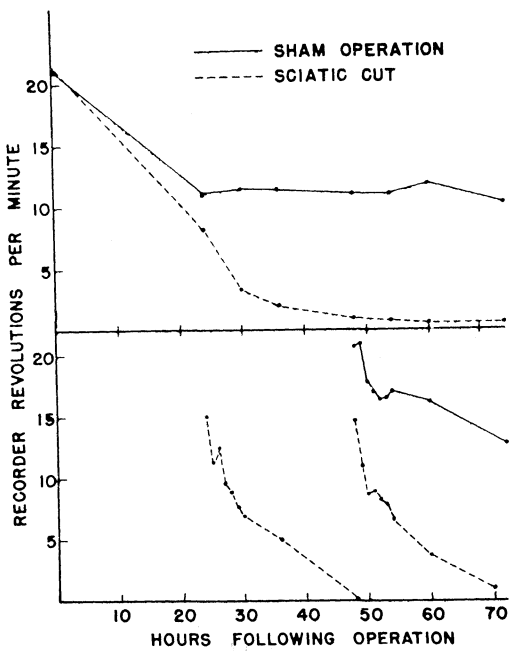

FIG. 1.

The effect of cutting the sciatic nerve upon the ability of the gastrocnemius muscle to respond to faradic stimulation.

The data on averages for rates of work per minute for all animals are summarized in Fig. 1.

Discussion. In these experiments the responsiveness of the gastrocnemius muscle was decreased below normal within 24 hours following the cutting of the sciatic nerve. The decrease occurred in those animals which were kept for 24 hours before stimulation was started as well as in the series which were stimulated continuously. The performance of work did hasten the "fatigue" of the denervated muscle, as is evident from the curves of average performance (Fig. 1) of those animals which worked continuously as compared with the performance of rats which were delayed for 24 and 48 hours following denervation.

The early effect of denervation upon the response of the muscle to direct faradic stimulation appears to be due to a rise in the threshold for adequate stimulation rather than an incapacity of the muscle to contract at all. When the intensity of the pulse was increased from 12 to 20 milliamperes at the end of the experiment the muscle contracted strongly again and sustained a high rate of work for several hours. The response was less than in the sham-operated control animals. In considering this effect it is important to recognize that our standard stimulus is optimal under ordinary experimental conditions. It will elicit an optimal amount of total work in normal animals during several days, although a temporary rise in rate of work can be induced by a more intense stimulus. A pulse of 20 milliamperes is sufficiently intense to cause tissue destruction within a period of a few hours.

The explanation of the early effect of denervation upon the ability of muscle to work is not known to us. The loss of muscular responsiveness may be due to metabolic changes caused by denervation possibly relating to the changes which result in muscular atrophy. An alternative explanation is that the dying of the nerve fibers removes an important excitatory mechanism from the

muscle. Although the response of muscle to direct stimulation has commonly been described as due to the direct excitability of muscle fibers, it seems possible that the spread of the electrical pulse through the entire muscle may excite the nerve fibers which innervate it to play an important role in eliciting the response of muscle.

Separate experiments (unpublished data) have been carried out to determine the effect of curare upon the ability of the muscle to sustain responsiveness to faradic stimulation. If it were possible to completely block the myoneural junction without unfavorable effects upon respiration and circulation, the structures involved in the response to the stimulus could be further elucidated. Normal rats were given continuous subcutaneous injections of curare for periods up to 48 hours In these experiments curare did not affect work output in any dosage which did not cause respiratory failure. It was also shown that doses which failed to cause respiratory symptoms also failed to completely block the myoneural junctions in the stimulated muscle.

Summary. Male rats (310 to 350 g) were subjected to direct faradic stimulation of the gastrocnemius muscle following the cutting of the sciatic nerve, and their work performance was compared to that of sham-operated controls. When stimulation was started immediately following the operation the rate of work of denervated muscle was normal for several hours but was depressed below normal by 24 hours and continued to decrease rapidly, whereas the control animals sustained a high rate of work. When stimulation was delayed for 24 and 48 hours following denervation, the initial contractions were subnormal and decreased rapidly during continued stimulation. When the intensity of the pulse was increased from 12 to 20 milliamperes, at the end of each experiment there was a striking recovery in the rate of work of denervated muscle but it remained below that of the sham-operated controls.

16238 P

Rodenticidal Action of 2-Chloro-4-Dimethylamino-6-Methylpyrimidine (Castrix).*

KENNETH P. DUBOIS, KENNETH W. COCHRAN, AND JOHN F. THOMSON.

From the University of Chicago Toxicity Laboratory and the Department of Pharmacology, University of Chicago, Chicago.

At the close of the European phase of the war it was revealed that German scientists had developed a new rodenticide (2-chloro-4-dimethylamino-6-methyl-pyrimidine) which was known as "Castrix." Since no data on the toxicity of Castrix were available in this country and no antidote against accidental poisoning in man and domestic animals was known the present investigation was undertaken to ascertain the toxicity of Castrix to several species, to examine its efficacy as a rodenticide, and to find an effective antidote for the treatment of accidental poisoning. The results of this preliminary study demonstrated the high toxicity of Castrix and the effectiveness of pentobarbital sodium as an antidote against Castrix poisoning.

Experimental. In all of the species examined Castrix produced symptoms typical of central nervous system stimulants. Convulsions occurred after a latent period of 15 to 45 minutes following either oral or intraperitoneal administration. The head retracted and the fore-legs and hind-legs were tetanically extended. After the tonic phase

* The work described in this paper was done under contract between the Medical Division, Chemical Corps, U. S. Army, and the University of Chicago Toxicity Laboratory. Under the terms of this contract the Chemical Corps neither restricts nor is responsible for the opinions or conclusions of the authors.