viving frog intestine strips is in accord with the findings of Cannon and Burket,[3] on the outstanding resistance of these cells in the cat intestine to pressure and low oxygen.

Since the cells of Auerbach in these surviving frog intestine strips showed little or no change until after the spontaneous activities ceased it seems probable that the Auerbach cells could have been functioning throughout the entire survival period of the muscle cells, *i. e.*, without additional evidence it would not be proper to consider the spontaneous movements of the surviving strips as entirely myogenic in origin in spite of the fact that the development of pyknosis in the muscle cell nuclei was correlated with the cessation of spontaneous movements.

9459

Loss of Potassium from Stimulated Frog Muscle.

W. O. FENN.

From the Department of Physiology, School of Medicine and Dentistry, The University of Rochester, Rochester, N. Y.

We have reported previously[1] that on stimulation rat muscles lose potassium in exchange for sodium, but that similar experiments with frog muscles showed no appreciable loss of potassium when the muscle was stimulated through its nerve. Other investigators[2, 3, 4] have had similar difficulty in demonstrating a loss of potassium with indirect stimulation of frog muscle. It now appears that a loss of potassium can readily be demonstrated in frog muscle if the nerve is stimulated with single shocks delivered at such a frequency that good contractions can be maintained for a period of 1.5 hours or perhaps less. If the muscle is continuously tetanized, the contractions fall off rapidly, not because of fatigue of the contractile mechanism but because of inhibition at the myoneural junction. Thus the potassium content, which seems to be related to the contractile mechanism rather than to the excitatory mechanism, is not affected.

The frogs *(Rana pipiens)* were usually anesthetized with 1 cc. of

3 Cannon, W. B., and Burket, I. R., *Am. J. Physiol.*, 1913, **32**, 347.

1 Fenn, W. O., and Cobb, D. M., *Am. J. Physiol.*, 1936, **115**, 345.

2 Mond, R., and Netter, H., *Arch. ges. Physiol.*, 1930, **224**, 702.

3 Mitchell, P. H., and Wilson, J. W., *J. Gen. Physiol.*, 1921, **4**, 45.

4 Ernst, E., and Csúcs, L., *Arch. ges. Physiol.*, 1929, **223**, 663.

20% urethane per 100 gm. body weight (otherwise etherized or decerebrated). The animal was fastened to a frog board, one Achilles tendon being cut and attached to an isotonic recording lever and the corresponding sciatic nerve being in contact with stimulating electrodes. After 1.5 hours of stimulation, both legs were clamped off, the gastrocnemius (and sometimes also tibialis plus peroneus) muscles removed, squeezed gently and blotted on filter paper to remove blood, and put into weighing bottles. Wet and dry weights (110°C. to constant weight) were obtained and the residue was transferred to platinum crucibles, ashed at 500°C. overnight, and analyzed for potassium. I am indebted to my assistant, Miss Doris M. Cobb, for the potassium analyses. The wet weights of the muscles varied from 0.59 to 1.15 gm. for the gastrocnemius, and from 0.27 to 0.57 gm. for the tibialis and peroneus.

TABLE I.
Stimulation of Frog Muscles at Varying Frequencies and Loads (per 100 gms. dry weight).

Exp. No.	Load gm.	Frequency per min.	Resting muscles H_2O cc.	Resting muscles K millimols	ΔH_2O cc.	ΔK millimols
1	5	16-51	440	48.4	+94	—1.8
2	5	51	415	41.7	+57	—1.1
3	5	73	356	45.1	+90	—3.4
	5	73	352	46.1	t +55	—4.0
4	5	97	362	44.5	+76	—2.3
	5	97	356	45.9	t +59	—3.7
5	5	166	392	40.2	+117	—2.4
	5	166	381	40.4	t +111	—2.4
6	5	322	399	43.0	+101	—0.9
	5	322	412	41.7	t +108	—1.2
7	5	3420	411	46.4	+72	—1.8
	5	3420	416	47.1	t +99	—3.8
8†	57	93	384	49.2	+197	—4.2
9	57	93	348	45.5	+151	—3.0
10*	57	93	420	49.7	+127	—2.2
11*	57	93	396	50.0	t +84	—2.9
Average, 1.5 hr. intermittent			390	45.3	+100	—2.6
Average, 10-30 min. continuous (from Fenn and Cobb[1])					+121	—0.3

†Decerebrate.
*Ether anesthesia.
t = tibialis and peroneus muscles; all other gastrocnemius.
H_2O and K calculated per 100 gms. of dry weight of muscle. All animals under urethane except as otherwise noted. The load is the tension on the muscle itself.

The results are reported in Table I. In 16 pairs of muscles from 11 frogs, without exception, there was a loss of potassium and a gain of water. For comparison, the average loss in our previous series is included in the table. If contractions are maintained for a sufficient period of time, there is evidently no difficulty in demonstrating a

typical loss of potassium in frog muscles, even with indirect stimulation.

At the higher frequencies listed in Table I, the contractions were continuous as in our earlier series but the loss of potassium was nevertheless evident. The greater loss observed in our present series as compared to the earlier series is not altogether due to the greater duration of stimulation, and maintenance of a muscle in a condition of "complete fatigue" by high frequency stimulation for 1.5 hours is not as effective in mobilizing potassium as intermittent contraction at lower frequencies. To show this we tried stimulating both legs of a frog at the same time, one leg at a low frequency so that it gave discrete twitches which were fairly constant in magnitude, and the other leg at a high frequency so that it gave a smooth tetanus which fell off rapidly in height. This frequency was usually 17/sec. but was varied somewhat in an effort to keep the contraction maximum. After 1.5 hours, the muscles were sampled and analyzed as before. The results (Table II) show a strikingly lower potassium content and a somewhat greater water content on the side where the contractions remained large, although the total number of stimuli delivered in the 1.5 hours was 17 times greater on the continuously stimulated side. The tension-time ($\int Tdt$) developed (above the initial tension of about 10 gm. in each muscle) was not accurately measured, but was certainly larger in the intermittently contracting muscle. Further experiments are necessary, however, to decide whether the loss of potassium is proportional to the performance of the muscle (tension-time) or to the degree of fatigue of the contractile mechanism proper.

TABLE II.
Continuous vs. Intermittent Stimulation in Frogs
(per 100 gms. dry weight).

	Water		Potassium	
	Continuous	Intermittent	Continuous	Intermittent
Exp. No.	cc.	cc.	m.-eq.	m.-eq.
1g	461	492	50.5	44.0
2g	537	568	50.0	45.0
2t	540	572	48.8	46.9

g = gastrocnemius; t = tibialis and peroneus. Frogs were under urethane. Contractions were recorded on a tension lever permitting considerable shortening. Initial tension about 10 gm. Duration of stimulation 1.5 hours.

There seems to be no direct conflict between these data and others previously published. Mitchell and Wilson[3] quote 2 experiments showing no loss of potassium due to indirect stimulation, but they used perfused muscles which might have already lost to the perfusate

all the mobile potassium from both muscles of the pair, and they stimulated only for 2 seconds out of every minute with 30 minutes out of every hour for rest. Mond and Netter[2] and Ernst and Csucs[4] also used perfused muscles. The former authors stimulated for 30 minutes only, while the latter used short tetani every second until fatigued, repeating this several times, but do not mention the total duration of the stimulation. Ernst and Takács[5] quote 3 experiments showing a loss of potassium from perfused muscle indirectly stimulated but ascribe this result to the use of high potassium in the perfusate which was supposed to have increased the permeability of the muscle, thus permitting loss of potassium. Our results show that a high potassium concentration outside the muscle is not necessary for the phenomenon. We have not tried to introduce the unnecessary complication of perfusions in order to see whether a loss of potassium in activity can still be demonstrated by suitable stimulation under these conditions.

Summary. Frog muscles which are kept twitching rhythmically for 1.5 hours with well maintained contractions show a consistent average loss of 5.7% of the potassium originally present and a gain of water.

9460 P

Effect of Benzedrine Sulphate on Stomach Activity and Emptying Time.*

KARL H. BEYER AND W. J. MEEK.

From the Department of Physiology, University of Wisconsin Medical School, Madison.

Benzedrine sulphate is used clinically for the relief of pylorospasm and the facilitation of fluoroscopic and roentgenoscopic examination. Meyerson and Ritvo[1] reported that the drug brought about satisfactory disappearance of spastic manifestations in 85% of their cases.

On the other hand ephedrine sulphate, a closely related sympathomimetic compound, in doses of 65 mg. was reported by Van Liere, Lough, and Sleeth[2] as prolonging the emptying time of the stomach from 72.8 to 118%.

5 Ernst, E., and Takács, I., *Arch. ges. Physiol.*, 1931, **228**, 690.

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1 Meyerson and Ritvo, *J. A. M. A.*, 1936, **107**, 24.

2 Van Liere, Lough, and Sleeth, *J. A. M. A.*, 1936, **106**, 535.