tween the initial innervation and the choline ester response has not been determined. More intimate morphological details of what occurs when the nerves make contact with the muscle fibers remain to be worked out. However, these findings suggest that the receptive substance may develop only after the nerves join the muscle fibers.

9458

Differential Survival in Isolated Strips of Frog Intestine.

CORNEALIA G. ELLIS. (Introduced by M. M. Ellis.)

From the Department of Physiology and Pharmacology, University of Missouri, Columbia.

As part of the problem of survival of isolated tissue it became desirable to determine what elements within the tissue itself are responsible for the failure of spontaneous activity. Data are presented here correlating the physiological activity as measured by spontaneous movements with the histological picture in surviving strips of small intestine taken from the common leopard frog, *Rana pipiens* Shreber. In all, 50 frogs were used and over 250 series of activity records taken.

Segments one to 2 cm. in length, either intact or split lengthwise along the greatest curvature, were cut from the upper portion of the small intestine and transferred to shallow glass dishes containing 5 cc. of Ringer's fluid, buffered to pH 7.4-7.5 with carbonates and phosphates. No bacteriostatic substance was used, but the Ringer's fluid was changed twice daily. The strips were held at 5°C. except for an 80-minute period each day when each strip was mounted, by means of threads attached at the time of its removal from the frog, in a warm chamber filled with Ringer's fluid at 37.5°C. During this 80-minute period a continuous record of all spontaneous movements of the longitudinal muscle fibers was taken on an electric kymograph.

Frog intestine under such conditions survived for long periods as compared with the reported survivals of other smooth muscle preparations. One group of 16 frogs strictly comparable in all details shows the range of survival found in these tests, the minimal survival being 143 hours, the average 180 hours, the maximal 239 hours. Thirty-one percent of the survivals exceeded 200 hours. The maximal survival as indicated by the spontaneous activity of these frog intestine strips, 239 hours, was twice the survival time for activity of isolated rabbit intestine reported by Gunn and Underhill,[1] and equalled the maximal survival of irritability in the *retractor penis* of the horse, as demonstrated by Sertoli.[2] Isolated cat uterus given treatment similar to that accorded the frog intestine survived only 143 hours, a period equalled or exceeded by even the less hardy frog intestine strips.

The spontaneous movements of the longitudinal muscle were of 2 types, namely, large slow contractions of varying speed and amplitude upon which were superimposed small contractions of approximately uniform size and very regular rate. From 210 records of 26 frog strips these small regular contractions were found to occur at intervals of about 10 seconds when the excised frog strip was held at 37.5°C. The persistence of these small contractions throughout the entire survival period at approximately a 10-second rhythm and the regularity of this rhythm in all of the preparations of frog intestine studied were striking. Two exceptions must be noted, however, namely, that immediately after removal from the body of the frog, when the tissue was presumably in shock, and during the last run before spontaneous activity failed entirely, the rate of the small contractions was definitely slower, the rhythm being 12 to 15 seconds or longer.

Histological studies of surviving preparations taken from the activity series and fixed in Bouin's fluid at various times after removal from the frog were made throughout these experiments. As histological controls other strips were prepared as if for an activity series, and given the same daily routine treatment except that these control strips were held continuously at 5°C. and were never attached to the recording apparatus. One of these controls was carried for 478 hours. The final analyses showed that the experimental conditions imposed upon the strips of the activity series did not alter materially the sequence of the histological changes in these surviving strips as compared with the non-active controls, although these changes seemed to develop a little more slowly in the controls. The data from the activity series are presented.

During the first 2 or 3 days of survival very little change in the histological picture was observed, although the granules of the nuclei, particularly of the smooth muscle fibers, tended to become slightly coarser after 48 hours. At approximately 72 hours all muscle layers showed an increasing affinity for eosin, the maximal

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[1] Gunn, J. A., and Underhill, S. W. F., *Quart. J. Exp. Physiol.*, 1914, **8**, 275.

[2] Sertoli, E., *Arch. ital. de Biol.*, 1883, **3**, 78.

staining reaction with this dye being obtained between 72 and 100 hours.

From 72 hours on the changes in the nuclei of the muscle cells were very evident. Each nucleus shrank, and wrinkled so that by the end of 150 hours of survival most of the muscle nuclei were definitely pyknotic and many were showing signs of fragmentation. After approximately 180 hours of isolation the muscle nuclei gradually lost their staining power, disintegrated, and disappeared from the preparation. By the end of 336 hours very little nuclear material remained in the muscle layers or throughout the strip, except a very definite line of pyknotic nuclei between the longitudinal and circular muscle layers, at the location of the ganglionic cells of the Auerbach's plexus.

A count of the nuclei per measured unit of cross-section of the longitudinal muscle layer showed that the disappearance of nuclei from the surviving strips could be divided into 3 phases. The first 240 hours of survival were marked by a steady but gradual decrease in the number of nuclei. Although during this period the muscle nuclei were undergoing definite degenerative changes, only about 5% of the nuclei actually disappeared per day. Near the 240-hour point a second phase developed lasting some 96 hours during which approximately 80% of the remaining nuclei disappeared and those nuclei which persisted became markedly altered, *i. e.,* this phase was characterized by rapid disappearance of nuclear material. From approximately the 336th hour of isolation to the final disintegration of the tissue the few nuclear remnants disappeared at the rate of about 1% of the original nuclear count per day.

As most of the surviving strips studied ceased spontaneous movements after 150 to 200 hours, and as the maximal survival was 239 hours, a correlation with the nuclear findings in the muscle cells suggests that the development of the pyknotic condition of the muscle cell nuclei which became general and widespread throughout the muscle layers between 150 and 200 hours was a determining factor in the cessation of spontaneous activity.

The survival of the cells of Auerbach's plexus in these isolated frog intestine strips was conspicuous. During the first 100 hours these ganglionic cells showed very little change, even their processes being well defined in properly fixed preparations. After 100 hours of survival the progress of nuclear change was much slower in the Auerbach cells than in the smooth muscle cells, the nuclei of these ganglionic cells being the last nuclei to disappear in the frog intestine strips. The persistence of the Auerbach cells in these surviving 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

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[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.