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Microscopy of Synapses of Non-Excised Frog Heart. (19636)

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The synapses of beating but excised frog heart have been studied by Fedorov(1), with the Gramenitsky preparation(2,3). This was made possible by stretching of the atria and sinus venosus, and dissecting away of adventitious tissue. These portions of the heart are then sufficiently translucent for microscopic study of the detailed structure of the synapses with the ganglion cells, which lie in these portions of the heart. It is also feasible, however, to subject the synapses to microscopy without removing the heart from the body or severing the vagi. A note limited to the procedure and apparatus required and the range of its applications will appear elsewhere. It may be noted here that they serve to stretch and transilluminate these portions of the heart with the organ *in situ*. Under these conditions, the terminations of the vagus and the ganglion cells on which they synapse are more viable, and display the following characteristics.

The ganglion cells. In a fresh preparation of the heart *in situ*, untreated except with Ringer's, and viewed with an oil-immersion objective and Ringer's in place of oil, the ganglion cells are concealed in opaque white capsules of connective tissue. The preganglionic terminals and end-feet are outlined as fine linear and vesicular elevations of the surface. After many hours, often up to 6 or longer, the opacity may diminish by itself, and the cell itself gradually becomes visible in detail. The cytoplasm then reveals a distinct granulation, but no cell membrane is ever to be seen. The nucleus presents itself centrally located, with its membrane and nucleolus both sharply defined. Eventually the nucleus moves to a side, very frequently in an *axofugal* direction. The axon frequently is bent on itself to approximate the border of the cell for a short distance. The connective tissue capsule remains visible in part, as a bracelet which closely encircles the cell. One or more fibroblasts are to be seen in the capsule, in addition to one frequently near the flexure of the axon.

On the other hand, in the *excised* preparation, the opacity of the capsule has often disappeared by the time of mounting, and if not, the translucency and enhanced cell visibility develop in a shorter period than in the non-excised heart. It is to be concluded from the time required to mount the excised heart, that morphologic deterioration of the cells begins within an hour of severance of the pregan-

glionic trunks and abolition of the circulation.

The Vagal Terminals and End-Feet. With emergence of cellular detail in the heart *in situ*, the presynaptic structures also become visible. The terminals of the vagus are extremely fine and can be discerned for short distances only. The end-feet are tiny spheroids and occur in clusters on the cell. Both structures retain their morphology for hours. Eventually many of the fibers and end-feet become invisible or disintegrate, while other end-feet become slightly enlarged and on some cells grossly swollen. In the *excised* heart, on the other hand, the visible fibers and end-feet are fewer by the time that the mounting is accomplished, and a slight enlargement of most end-feet has already taken place. It is to be concluded that vagal terminals and end-feet, like the ganglion cells, begin to deteriorate within an hour of sectioning of the trunk and abolition of the circulation.

Enhancement of Visibility by Acidification. The enhanced visibility of cellular and presynaptic structures which has been described can be produced at once, as observed by Fedorov(1), by a drop of acetic or other acid of around pH 3.5. The effects do not reverse themselves. They are reversed by a drop of dilute alkali, however, which produces a pronounced opacity which gives way to translucency once more on reapplication of acid. The gradual enhancement of visibility without acid, in preparations untreated except with Ringer's, is presumably the result at least in part of accumulation of acid metabolites. The acidity would bring micelles of protoplasm closer to their isoelectric points, reducing their lyophilia and precipitating more of the coacervate out of the dispersion medium.

Functional Integrity and Morphologic State. Stimulation of the vagus is effective in diminishing or arresting the heart beat even when the visible end-feet are in the stage of slight enlargement, and ganglion cells mostly in the stage of enhanced visibility. In a subsequent stage of deterioration, the end-feet are very few and the cells are tenacious of supravital stains, which earlier they reject except for cytoplasmic granules. A functional integrity of synapses persists until most of the end-feet and cells are in these states of more advanced deterioration.

Microscopy of Synaptic Transmission. Changes in the appearance of the synapses can be observed under the microscope during tetanization of the vagus which results in inhibition of the heart beat. The visible changes are *not* instantaneous, but appear in from 5 seconds to a minute. The end-feet become more glistening and seem as if distended with or exuding clear liquid. They sometimes become yellowish, an effect which can sometimes also be produced by a drop of acetylcholine. The ganglion cells develop a greater clarity of detail. On cessation of the stimulation, the effects tend to subside partially or completely. The observations confirm in general and amplify those of Fedorov on the *excised* heart(1).

Summary. The synapses of beating, non-excised frog heart are amenable to microscopy with the vagi intact, and reveal a number of differences from the synapses of beating heart which has been excised. The terminals and end-feet of the vagi, and the ganglion cells on which they impinge, can be observed to pass through two stages of morphologic deterioration. The vagal effect can be produced until the second stage predominates in the preparation. Synaptic transmission from tetanization of the vagus is followed by visible changes in both the pre- and the postsynaptic structures.

1. Fedorov, B. G., *Trav. du Lab. de Recherches biol. de l'Université de Madrid,* 1935, v30, 403.

2. Gramenitsky, M. I., *Z. f. Zellforsch. u. Mikr. Anat.,* 1934, v21, 580.

3. Gramenitsky, M. I., *Abderhaldens Handbuch der biol. Arbeitsmethoden,* 1935, v5, 953.

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