Ultracentrifugation of Rat Intestinal Mucosa[1]

During the course of studies on the mechanism of iron absorption in rat intestinal mucosa, the fractionation of mucosal tissue was attempted utilizing the scheme of SCHNEIDER and HOGEBOOM for rat liver homogenates[2]. It was found, however, that modifications of their procedure had to be made before a satisfactory separation of the mucosa could be accomplished.

The mucosa was scraped from the whole of the intestine and homogenized in 0.25 *M* sucrose with a ground glass hand homogenizer. These operations were performed in a cold room at 4°C. Fractionation was accomplished with a Spinco Model L Preparative Ultracentrifuge using the following concentrations of sucrose in separate experiments: 0.25 *M*, 0.44 *M*, and 0.88 *M*; 0.88 *M* sucrose plus 0.0018 *M* calcium chloride; and 0.88 *M* sucrose plus 3 U/ml of heparin.

Samples taken from the particulate fractions isolated from the media described above were examined by light, phase and electron microscopy. All fractions revealed gross contamination. The nuclei were markedly agglutinated and contaminated with trapped cytoplasmic particles (mitochondria and microsomes). SCHNEIDER and HOGEBOOM found that as much as 10% of the mitochondria were lost in this manner from rat liver[3]. With the use of 0.0018 *M* calcium chloride in 0.34 *M* sucrose, HOGEBOOM et al.[4] were able to prevent nuclear agglutination. In our experiments the addition of calcium chloride effected a clean separation of nuclei, but caused increased agglutination of cytoplasmic particles as reported by SCHNEIDER[5]. After isolation of the nuclear fraction, 0.0018 *M* ethylenediamine tetraacetic acid (EDTA) was added to the supernatant in order to remove the calcium ions. This procedure failed to decrease the agglutination of the mitochondria and microsomes. Fearing the loss of iron through chelation with increased concentrations of EDTA, the use of calcium chloride and EDTA was discontinued.

Because of its propensity for maintaining the dispersion of colloidal particles, heparin was tested in the fractionation scheme. With as little as 3 U of heparin/ml of 0.88 *M* sucrose, the isolation of homogeneous fractions was accomplished. The nuclei were well dispersed with almost

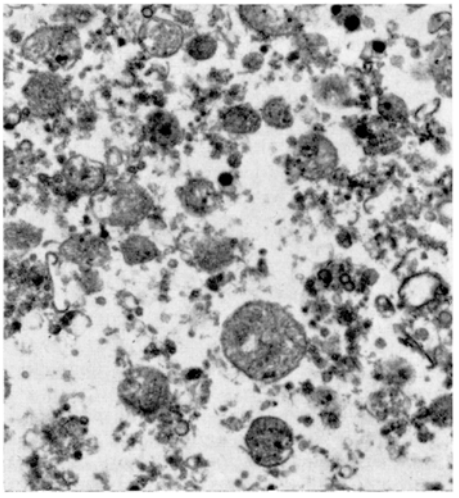

Fig. 1. Electron photomicrograph of the mitochondrial fraction isolated in 0.88 *M* sucrose. × 10,500.

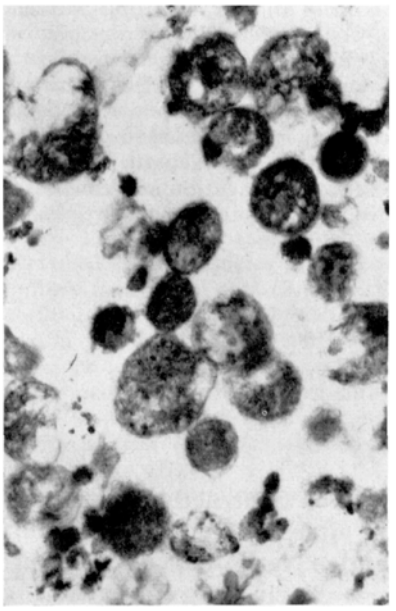

Fig. 2. Electron photomicrograph of the mitochondrial fraction isolated in 0.88 *M* sucrose with heparin added (3 units/ml). × 17,000.

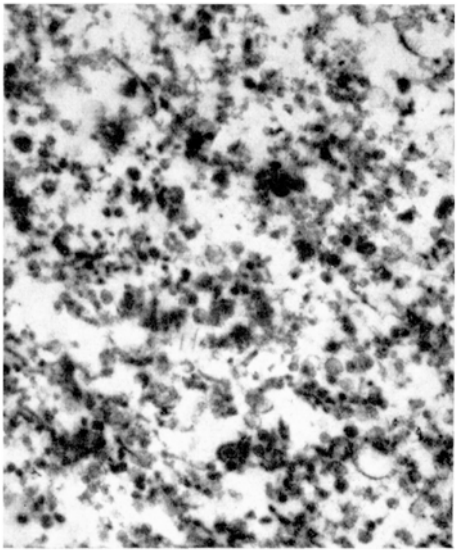

Fig. 3. Electron photomicrograph of the microsomal fraction isolated in 0.88 *M* sucrose with heparin added (3 units/ml). × 12,000.

[1] This work was supported by USPH Grant No. 5 T 1 HD-18-04, Child Health and Human Development Branch.

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[3] W. C. SCHNEIDER and G. H. HOGEBOOM, J. biol. Chem. *198*, 155 (1952).

[4] G. H. HOGEBOOM, W. C. SCHNEIDER, and M. J. STREIBICH, J. biol. Chem. *196*, 111 (1952).

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complete absence of contaminating mitochondria and microsomes. As seen with electron photomicrographs (RCA Model EMV-3G electron microscope using a 1% buffered osmium tetroxide fixative), the mitochondrial and microsomal fractions were found to be homogeneous enough so that experiments could be performed.

It is felt, therefore, that heparin can serve as a useful tool during ultracentrifugation experiments for the separation and isolation of subcellular particles.

Résumé. Nous avons présenté une méthode de fractionnement et d'isolement des particules subcellulaires de la muqueuse intestinale du rat. L'héparine permit l'isolement de fractions relativement homogènes.

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STUDIA NOVA

Spontaneous Contractions and Bioelectric Activity after Differentiation in Culture of Presumptive Neuromuscular Tissues of the Early Frog Embryo

Tissue culture has recently been used to study the capacities of isolated regions of the amphibian neural plate for physiological development[1]. This experimental method has also been successfully applied to studies of the bioelectric activity of fragments of spinal cord[2,3] and cerebral cortex[4,5] isolated from later stages of mammalian (and avian) embryos and neonates. The present report describes electrophysiologic experiments on cultures of frog neurula tissue, explanted together with presumptive axial muscle, after development in vitro of spontaneous twitching. The data indicate that the characteristic, spontaneous bursts of muscle contractions in these explants may be triggered by endogenous, rhythmic neural activity.

The tissues were obtained from neurulae of *Rana pipiens* (stages 13–17, Shumway). The desired region of the neural plate, usually the prospective upper spinal cord but occasionally also the prospective hindbrain, was excised together with the underlying chorda-mesoderm. The explants (about 0.5 mm^3) were cultured on a glass, or collagen, surface in a small petri dish containing several ml of a balanced salt solution (Barth's 'X') with added globulin[6]. The cultures were examined regularly for muscular activity, which was then carefully observed and described. For bioelectric recordings, the culture coverglass was inverted so as to form the roof of a small moist chamber. The chamber was mounted on a microscope stage and micromanipulators were used to position microelectrodes at various sites in the tissue[3,7]. A given culture was studied electrically only once, and both the spontaneous activity and that evoked by electric shocks were recorded over a period of several hours (with differential-input preamplifiers and an oscilloscope).

Undisturbed cultures survived and showed muscular twitching for up to two weeks without added nutrients or change of medium. It should be emphasized that the exogenous nutritional requirements of early embryonic amphibian tissues are much simpler than those of mammalian explants since the individual cells still contain abundant endogenous yolk reserves. The explanted fragments remained as a thick mass of cells, but extensive outgrowth of muscle and connective tissue cells, and of nerve fibers, often occurred. Numerous isolated cells also grew near the main mass and were often attached to nerve fibers, which generally branched extensively from that point. In five instances an outlying single cell or small cluster, visibly connected with nerve fibers, was seen to twitch. In two of these, the twitches were synchronous with bursts of muscle contractions in the main mass, while only intermittent single twitches were seen in the other three.

Spontaneous twitching of the explant developed in more than half of the cultures prepared with neural plate and the underlying layer. This was never seen in numerous cultures of chorda-mesoderm alone despite the development of numerous muscle fibers, which could be made to twitch by electric shocks. Spontaneous activity was typically in the form of synchronous contractions of some or all of the muscle of the explant, usually with temporary desynchronization of single fibers here and there during a burst of twitches. The earliest activity seen in explants of prospective spinal cord was, characteristically, a single twitch at intervals of $^1/_2$ min or more. This occurred between the 4th and 7th days in vitro when the tissue was isolated early in neurulation (stages 13, 14), but as early as the 2nd and 3rd days if isolated somewhat later (stages 15–17). Prospective hindbrain developed relatively rapidly, for all of those cultures (isolated at stage 14) showed frequent activity on the 6th day, when half of the cord explants showed no activity or only an occasional twitch. In every case but one where single twitches had been observed to occur, *bursts* of contractions were seen on the following day. (The single exception showed only occasional single twitches for several days, and then stopped all muscular activity; a few preparations were also seen to revert to this pattern shortly before cessation of all activity.) Three of the 36 active preparations studied developed a pattern of almost continual twitching, at the same average frequency as that which occurred in the short bursts.

Burst activity in all of these explants can be characterized as follows: (a) twitches occurred at a rate of about

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