Nature of the Striated Muscle Fibre Membrane

At the present time, considerable confusion exists as to what should properly be called the sarcolemma, or true muscle fibre membrane. Long[1] defined the sarcolemma as a bipartite structure consisting of an inner layer, the true plasma membrane, and an outer layer composed of reticular fibrils. Meyer and Bernfeld[2] concluded that the sarcolemma is a collagenous-type protein. Jones and Barer[3] failed to find evidence of a collagenous or fibrillar structure.

Cytochemical tests have been carried out on the sarcolemma of isolated muscle fibres of the frog, *Rana pipiens*. In the process of teasing out fibres from various leg muscles, retraction clots were formed in the protoplasm. These clots shrank away from the sarcolemma, thus exposing large areas of the free membrane. The Withrow Morse[4] test for collagen was applied to fifty muscle fibre fragments as well as to entire muscle and bundles of fibres. The sarcolemma in the isolated fibre fragments invariably gave a negative response for collagen. In the whole muscles and bundles a positive reaction was obtained. In the second test for collagen (Chittenden[5]) muscles were boiled in a solution containing dilute sodium hydroxide and nitric acid. Numerous wrinkles, crevices, and folded edges appeared in the sarcolemma. The latter appeared to be festooned along the outer edges of the fibre. These festoon-like areas probably resulted from the swelling effect of the dilute acid. The third test, that of Barer[6], involved digestion of the fibre material in a 0·15 per cent solution of crystalline trypsin at *p*H 7·8. As a control, ligaments, tendons and other collagenous tissue were used. After digestion for four hours the sarcolemma had ruptured and gone into solution. The control tissues were unaffected by trypsin, even after eight hours in the enzyme solution. The xanthoproteic and ninhydrin reactions for protein were applied to the sarcolemma. A positive result was obtained in both tests. In the xanthoproteic reaction, the colour intensity of the sarcolemma decreased as the concentration of the nitric acid was lowered. Lipids were tested for by means of nile blue sulphate and sudan III dyes, with alcohol–ether extracted fibres serving as controls. Positive tests for lipid in the sarcolemma were indicated in both cases.

Bairata[7] regards the sarcolemma as a network of collagen fibres imbedded in a homogeneous colloidal matrix. He states that he has seen fine fibrils within the sarcolemma under polarized light and dark-ground illumination. This could not be substantiated here, nor could fibrils be seen using a phase-contrast microscope. What Bairata possibly considered a collagenous network in the membrane may in reality be the areolar connective tissue investing the individual fibre. Meyer and Bernfeld[2], using the dye methyl red, and Reed and Rudall[8], using the electron microscope, appear to have demonstrated the presence of collagen in the sarcolemma. The latter authors were able to demonstrate collagen fibrils; however, when the optical level of the true sarcolemma was reached, they found it to have a dotted, rather than a fibrillar, make-up. Barer[6] attempted to digest isolated portions of sarcolemma with the enzymes hyaluronidase and collagenase, but without any obvious effects. Jones and Barer[3] failed to find any evidence of a collagenous or fibrillar structure. They found, as did Reed and Rudall, numerous small dots present within the sarcolemma.

Typically, in the present investigation, a positive reaction for collagen in the sarcolemma was always obtained when whole muscle or bundles of fibres were used. When individual fibres, free of endomysium and other connective tissue, were tested, collagen was invariably absent. Furthermore, the sarcolemma always gave positive tests for protein. Unlike collagen, it proved digestible by trypsin. These facts, together with the positive reactions for the presence of lipids, suggest that the sarcolemma is a protein, possibly a lipoprotein. This type of chemical constitution is in agreement with that of the classical object of plasma membrane chemistry, the mammalian erythrocyte envelope.

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Transamination in the Human Tooth

In previous communications[1,2] it was shown that the presence of free aspartic and glutamic acids could be demonstrated in carious but not in sound dentine. With the view that a knowledge of the metabolic processes in the sound tooth might aid in an understanding of the caries process, it was decided to investigate whether the sound dental tissues exhibited any enzymatic activity towards aspartic and glutamic acids as substrates.

Using a paper chromatographic method[3], it has been demonstrated that a transaminase occurs in the human tooth in dentine and pulp tissue. This enzyme catalyses the reaction:

$$\text{L}(-)\text{ aspartate} + \alpha\text{-ketoglutarate} \rightleftharpoons \text{L}(+)\text{ glutamate} + \text{oxalacetate} \quad \ldots (1)$$

Weaker activity was also demonstrated for the reaction:

$$\text{L}(+)\text{ alanine} + \alpha\text{-ketoglutarate} \rightleftharpoons \text{L}(+)\text{ glutamate} + \text{pyruvate} \quad \ldots (2)$$

Owing to the very real technical difficulties involved in working with such small amounts of starting material, it has not been possible as yet to isolate relatively pure active preparations of the enzymes. However, anaerobic incubations using crude extracts and tissue suspensions have been made, and preliminary quantitative studies carried out successfully. The optimum *p*H for reaction 1 is 7·5 and Q_t values for pulp tissue are in the order of 10–12. Studies on the rate of glutamic acid formation, compared with the associated aspartic acid decrease, indicate a greater formation of the former than can be accounted for by loss of aspartic acid. This problem is at present under investigation.