RETICULAR ORGANIZATIONS WITHIN THE STRIATED MUSCLE CELL

An Historical Survey of Light Microscopic Studies

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In a review of the fine structure of striated muscle, Barer (1948) writes:

"Relatively little interest seems to have been taken in the problems of muscle structure in the years between 1900 and 1930. The old observers had attacked the problem with such energy and in so many ways that there seemed relatively little left to do."

While it is true that the present century inherited from the last many basic concepts of muscle structure recognized today as valid, the study of this subject by no means flagged, since, along with the foundations provided by Bowman, Kölliker, and others, there came a welter of controversy and confusion. Many such controversies stemmed from the varied interpretation of techniques of observation, especially of staining methods, but of perhaps greater importance was the conceptual confusion arising out of the comparison between muscle and other cells. Terms which now have a rather precise structural connotation—Golgi apparatus, reticulum or ergastoplasm, sarcosome or mitochondrion—have all had a long history, but for each term that has survived, a score have passed out of use. Consider, for example, the term *mitochondrion*. Cowdry, in 1921, listed thirty other names which he recognized to be synonymous, in use before the general term was introduced by Benda in 1897, and Cowdry completed this list with twenty-three further synonyms and seventeen terms used to suggest origin, behavior, and function, coined after the acceptance of the mitochondrion as a universal cell organelle.

It is essential that, in attempting to trace the development of concepts of muscle structure, parallel work on other tissues be considered, because such a comparison was inherent in the original observations and descriptions of those who first investigated the organization of cells. As Bennett (1955, 1958) points out, we owe many of the current basic concepts of muscle structure to the work of Bowman, Brücke, Kölliker, and others, but only quite recent work has confirmed the essential validity of their ideas, and at the turn of the century there was scarcely a single aspect of muscle organization that was not entirely controversial. We shall not review or even mention all these controversies, but rather shall trace the origin and ultimate resolution of just one, concerning the nature of the sarcoplasm, and show that workers in the early years of this century by no means ignored or abandoned the unfinished lines of approach inherited from the last.

By 1900, certain basic concepts of muscle structure were already widely though not universally accepted. The muscle cell was often envisaged as consisting of a fibrillar and extrafibrillar phase, the former being contractile, and the whole being bounded by a membranous sheath or sarcolemma. Beyond this point, little agreement existed. The characteristic periodicity of striated muscle had attracted much attention, but agreement had been reached neither on the nature nor on the significance of the bands, nor on the manner in which band pattern and density changes are related to contraction and relaxation of the fiber. It was not clearly established whether the bands were restricted to the fibrils or were part of an extensive system, linking together the fibrils throughout the fiber by corresponding transverse sarcoplasmic membranes. Another problematical component was the variable population of sarco-

plasmic particles and granules; their nature and their spatial and functional relation to the fibrils and to the remainder of the sarcoplasm received attention but no uniform interpretation. Finally (in this incomplete list), it had been suggested several times, before 1900, that the sarcoplasm of the muscle cell either constituted or contained a reticulum or series of reticula; and in this volume is reprinted a translation of what is undoubtedly the best light microscopic description and assessment of this somewhat elusive component of striated muscle, originally published by Veratti in 1902.

Components or organelles recognized as being typically or invariably present in a wide variety of cells were sought in muscle, as it was generally assumed that the latter was essentially comparable with other cells, albeit extensively modified. This guiding concept was hampered by the fact that many structural details were near or beyond the limit of resolution of the light microscope, as well as by the limitations set by methods of preparation and visualization. Not only were many of the latter somewhat capricious (*e.g.*, metallic impregnation), but their specificity was unknown though often assumed, a fact responsible for much of the early controversy on the subject. The object of this paper is to select one structural detail, namely reticular organization within the muscle cell, and briefly to analyze the controversies which surrounded it; and incidentally to show, in one example, how the electron microscope not only makes possible the observation of the cell at a new level, but also lends new significance and clarity to observations made by the pioneers of cytology.

One of the most involved, and at the same time one of the most intriguing historical problems in biology lies in tracing the development of concepts of cellular organization. Though it would not be true to say that the study of cytology began with the advent of microtomy, the study of specially treated sections provided the first universally applicable basis for the comparison of one cell with another at the optical microscopic level. Today, the cytologist may describe a cell, often using terms coined nearly a century ago, and although the minutiae of his description may change after he has read the current set of journals, his account of the nucleus, cell membrane, reticulum or ergastoplasm, Golgi apparatus, and the like may primarily be a structural one, couched in terms of "membranes," "particles," "vesicles," "filaments," "fibrils," and so on. However, the transposition of many of these terms from light to electron microscopy was a great deal smoother than was their original formulation and acceptance, and, furthermore, care must be taken in equating early descriptions and definitions with present-day concepts. This is nowhere more evident than in the instance to be considered here: the taxonomic history of the Golgi apparatus of the muscle cell, and the "sarcoplasmic reticulum."

In this paper an attempt has been made to unravel a somewhat tangled subject. The illustrations provided by the early light microscopists often afford a clearer indication of their concepts (in the light of present-day knowledge) than does their accompanying text, and for this reason many illustrative examples have been reproduced here. As these are often considered and interpreted more fully in the figure legends than in the text, it may be of value to the interested reader to begin by glancing through these, before continuing with the main body of the paper.

EARLY CONCEPTS OF MUSCLE STRUCTURE

It has long been recognized that striated muscle is composed of two distinct phases, but the recognition by Bowman (1840), Brücke (1858), Kölliker (1866 and 1888), Rollett (1888) and others of the existence of contractile fibrils, embedded in a matrix, was not accepted without dispute. In particular, it was often supposed that the fibrils were artifactitious, *i.e.*, the product of coagulation by the fixative of a semifluid protoplasm, which was believed to constitute the matrix of the fiber, surrounding various series of relatively solid reticula. Among those who subscribed to this view were Gerlach (1877), Retzius (1881), Bremer (1883), Melland (1885), Marshall (1888, 1890), Carnoy (1884), Van Gehuchten (1886, 1888), and Cajal (1888, 1890), but despite the unanimity among these authors on this point, there was much disagreement among them on the question of the nature and function of the reticular framework they described.

Gerlach imagined that the postulated fluid matrix was contractile, and the framework elastic, and believed that the longitudinally disposed protoplasmic elements he observed within the fiber (Fig. 12) were connected with a "mantle" of nervous filaments surrounding the fiber and

continuous with the end plate nerve supply;[1] Retzius (1881) and Bremer (1883) likewise, though in a more cautious manner, implicated the network in impulse conduction. However, although both of these last authors described "networks" within the muscle fiber (Figs. 2, 15, and 16), it should be stressed that they considered these to be prolongations or protoplasmic trabeculae derived from the "muscle corpuscles" (actually the nuclei of the fiber) rather than the two components envisaged by Gerlach and others. They thought that the contractile element consisted of a reticulum of liquid-containing tubules, embedded in a more solid elastic matrix. Marshall, on the other hand (Figs. 3 *a*, 3 *b*, and 4), supposed that the longitudinally disposed elements of the complex reticular network were contractile and connected to the nerves via the elastic transverse components which were responsible for relaxation of the fiber, and stated that he would "only describe muscle as being striped when the striation is due to the presence of the intracellular network, described by Retzius, Bremer, and Melland." The last mentioned of these authors described intracellular longitudinal and transverse networks in a variety of vertebrate and invertebrate muscles (Fig. 1). He regarded the former network as "more or less separating the muscle-fiber into compartments being linked together by the longitudinal components which in section afford the appearance of striated fibrils"; and, he continues:

"The matrix, or substance which lies in the interstices of the network is of far greater bulk than the network. It is homogeneous throughout; nevertheless it may be looked upon as being partially divided into columns or fibrils by the longitudinal bars of the network, and particularly into discs—the contents of the muscle compartments—by the transverse networks. By the action of spirit the matrix becomes split into fibrils."

Carnoy favored the view that protoplasm consists of two chief elements: a fibrillar "reticulum" and a fluid matrix or "enchylema" described as both plastic and granular, filling the interstices:

"Qu'on se représente une éponge délicate dont les travées seraient remplacées par de simples trabécules, d'une extrème minceur, et dont les cavités seraient hyalines et visqueuses, parsemées de granules, et l'on aura une idée assez exacte, quoique très grossière de la masse protoplasmatique."

In the reticulum were thought to reside the properties of "contractility" and "irritability," considered by Carnoy to be characteristic of all living material, and the extension of this concept to include the postulated reticulum of the muscle cell was thus entirely logical. Indeed, the comparison was thought to be so perfect that Carnoy believed he had observed the actual transformation into muscle of cells of the gut of the water beetle *Hydrophilus* (Fig. 5), and it should be noted that accounts of supposed muscle reticula by several early athors, and notably by Van Gehuchten, were constructed in support of Carnoy's general hypothesis.

Van Gehuchten (1886, 1888) was the most painstaking proponent of the "fluid matrix school," and in concluding the latter work he summarizes his concept of muscle structure and its correspondence with other types of cell (Figs. 6 to 10) as follows:

"Le reticulum musculaire est la partie la plus importante.
"1. C'est en lui que réside la proprieté qui caractérise spécialement les cellules musculaires, la con-

[1] The morphological relation between the muscle fiber and its motor nerve supply was interpreted in a different way by Openchowski (1883) and by Thanhoffer (1882) (Fig. 11). While both of these authors allowed a *continuity* between nerve and muscle elements, the former described a neural plexus of fibers passing directly to the "muscle cells" ("corpuscles" of Bremer or, as they are now recognized to be, the muscle nuclei). The latter author, at least for insect muscle (see Fig. 13), described branches within the axis cylinder of the nerve (the axon sheath) which appeared to flow into or become continuous with the "lines of Krause" (the Z bands). This supposed continuity between nerve and muscle components was opposed by Kühne (1887).

These descriptions, while of historical interest as attempts to demonstrate the morphological pathway for impulse conduction within the fiber, and although they purported to demonstrate reticular or trabecular organization within striated muscle, do not in any way correspond to descriptions of the definitive sarcoplasmic reticulum of Veratti. It may be pointed out that electron microscopic examination of vertebrate and invertebrate myoneural junctions have so far failed to provide an instance in which the distribution of presynaptic elements corresponds to a repeating pattern either of the sarcoplasmic components or of the contractile material of the fiber.

tractilité; pendant la contraction, l'enchylème myosique ne fait que suivre, d'une manière toute passive, les mouvements du reticulum.

"2. Il forme ainsi la partie essentielle du muscle, celle sans laquelle la fibre musculaire ne peut exister. On pourrait conçevoir un élément musculaire sans myosine, mais on ne saurait admettre une fibre musculaire sans reticulum.

"Cette façon de comprendre la structure musculaire identifie la fibre striée avec la cellule ordinaire. En effet on y trouve également une partie organisée ou reticulum, et une substance de remplissage ou enchylème, il n'y a de différence que dans le mode d'arrangement du premier et dans les substances qui peuvent entrer dans la constitution du second. C'est donc à bon droit que l'on a défini la fibre musculaire striée [*quoting from Carnoy, 1884*]: 'une cellule ordinaire dont la reticulum s'est régularisé, et l'enchylème chargé de myosine.'

"Ajoutons que dans la fibre musculaire, comme dans toute cellule d'ailleurs, le reticulum forme la partie contractile, qui est siège de tous les mouvements physiques. *Au lieu d'affirmer avec Engelmann: 'il ne peut y avoir de contractilité sans éléments biréfringents,' nous dirons: il ne peut y avoir de contractilité sans reticulum isotrope.*"

Even Van Gehuchten, however, conceded that the fibrils of "dissociable" (Von Siebold) flight muscles of certain insects were present in the *living* fiber (1888) and not merely as coagulation artifacts in fixed tissue, and that they thus differed fundamentally from all other types in which the "fibrilles accidentelles" were thought merely to represent columns of coaglated enchylema. It is interesting to note that this anomaly was not considered by Van Gehuchten to invalidate his general conclusion in any way, since one of the basic tenets upon which he based his schema was that the *typical* fiber could not be separated, in the fresh state, into preexisting component fibrils.

Mingazzini (1888) proposed an ingenious (albeit erroneous) compromise, in which he attempted to reconcile the "fibrillar" and "reticular" models of muscle structure. He considered that fibrils preexisted in the living fiber, not as solid rods but as cylindrical membrane-bound tubes filled with viscous enchylema, and that when this substance was supposedly extracted (as in many of Van Gehuchten's preparations) the appearance of longitudinal elements of a reticulum represented the closely applied membranes of adjacent tubes; that the apparent filaments were merely optical sections of pairs of these membranes. In addition, he believed that during fixation the enchylema within the tubes became coagulated locally in the "dark band" and at the edge of the "membrane of Krause," thus giving the fiber its characteristic striated appearance.

Of the very few accounts of muscle reticula which allowed the existence of contractile fibrils in the living cell, and not merely as artifacts in fixed tissue, may be mentioned those of Thin (1874, 1876) and Haswell (1890). The former described, but did not illustrate, transverse elements which were held to be related in an intimate fashion or perhaps to be equivalent to the striation of the myofibrils; the descriptions of Haswell include a longitudinal component and a transverse membrane or network (Krause's membrane), running partly in the interspaces between the fibrils, but also across each isotropic band *through the substance of the fibrils themselves;* evidently the Z band of modern terminology.

Bütschli and Schewiakoff (1891) examined the structure of insect and crustacean muscle, and concluded that the originally homogeneous protoplasmic matrix of the fiber became transformed, upon fixation, into a reticular array of filaments (Figs. 19 to 21) surrounding the contractile fibrils, which were not considered to be continuous but rather to be composed of short columns ("Säulchen," "Platten") aligned end to end and joined to one another by a second meshwork running through the isotropic regions. These meshes defined by the filaments were described as irregular, and bear little resemblance to those of a definitive sarcoplasmic reticulum.

Although its significance was by no means generally appreciated at the time, a most important contribution to a solution of this problem had been made in 1888 by Rollett,[2] to whom we

[2] It is refreshing to find in addition, in the writings of Rollett, a plea for recognition of the value and accuracy of the work of many of those who first investigated muscle structure; work which was later all too often ignored or discredited:

"Zum Schlusse dieser kritischen Gänge möchte ich noch anführen, dass es mir beim Studium der umfangreichen Literatur der quergestreiften Muskelfasern immer den grössten Unmuth erregt hat, wenn ich gesehen habe, wie so oft alle Errungenschaften, welche wir Schwann, Bowman, Brücke, Cohnheim, Kölliker, Engelmann u. A. in der Erkenntniss des Baues der quergestreiften Muskelfasern verdanken, leichten Sinnes völlig über Bord geworfen werden, weil ich schon lange die Ueberzeugung habe, dass in

owe the term and the concept of "sarcoplasm," and who was able, on the basis of his own observations and conclusions, to criticize objectively the views of several of his contemporaries who had described so-called "reticula" within the striated muscle fiber. He considered that the images obtained (notably by gold impregnation methods) by Van Gehuchten and others displayed merely the sarcoplasmic packing material; that the existence and disposition of fibrils imposed a complementary honeycomb-like arrangement upon the sarcoplasm. By comparing fibers variously stained with gold chloride, hematoxylin, and osmium tetroxide, Rollett obtained selective coloration of either the sarcoplasm or the fibrils; a result especially striking in muscles unusually rich in the former, such as those of the sea horse *Hippocampus* (Figs. 28 to 32). His views are embodied in the following passage:

"Viele von den an die Existenz eines solchen Netzwerkes geknüpften Annahmen erscheinen gezwungen und einem einheitlichen und allgemeinen Verständnisse der bei verschiedenen Thieren auftretenden morphologischen Verschiedenheiten der Muskelfasern hinderlich, während durch meine im Eingange skizzirte Darstellung des Muskelbaues ein solches umfassendes Verständniss desselben vermittelt wird.

"Die Bilder, welche die genannten Autoren auf Fadennetze im Muskel beziehen, kommen nur durch die besondere Anordnung des Sarkoplasmas im Muskel zu stande, welches im Allgemeinen in Form eines Wabenwerkes die gegliederten Muskelsäulchen umgiebt.

"Ob das in solcher Weise angeordnete Sarkoplasma als solches noch eine feinere besondere Structur besitzt, die etwa mit der feinen netzartigen oder schwammigen Structur zu vergleichen wäre, welche man als 'Zellstructur' am Protoplasma nachzuweisen versuchte, müssen erst noch weitere Untersuchungen lehren, was ich hier, um künftige Missverständnisse zu vermeiden, den fälschlich angenommenen Fadennetzen im Sinne Melland's, Marshall's und Van Gehuchten's gegenüber noch besonders hervorheben will."

Rollett's explanation of muscle organization appears to have met with little initial enthusiasm, formulated as it was at a time when the views of Van Gehuchten and his school held considerable sway, but one of his few supporters was Schäfer (1891). Schäfer agreed that in fresh muscle treated with gold chloride and subsequently with formic acid, the sarcoplasm alone is stained while the sarcostyles (fibrils) remain colorless, in which case "the often described appearance of a network [*in transverse sections*] is obtained."

The requirements of the proviso expressed in the passage from the work of Rollett cited above, that the sarcoplasm (in his sense) might yet prove to contain further protoplasmic differentiation, were met by Veratti and discussed in his 1902 memoir.[3] This work not only provides us with excellent illustrations of reticula in many types of muscle, but is especially valuable for the precise terminology and definition it contains, which was employed as a basis for the critical assessment of earlier work. Veratti reiterated the objection made by Rollett to the views of Melland, Marshall, and Van Gehuchten, and similarly concluded *that the so-called reticula of these authors represented not a special differentiation within the sar-*

den von jenen Forschern aufgestellten Lehren vieles enthalten ist, was die directen Anknüpfungspunkte für einen erfolgreichen Ausbau der Histologie der quergestreiften Muskelfasern darbietet."

[3] The review published by Motta-Coco in 1901 is of considerable interest here, as it expresses concisely the degree of confusion which existed concerning the existence or status of supposed reticular systems in striated muscle in the period immediately prior to Veratti's work. The author, having surveyed the work of half a century, concluded:

"Die kurze Aufzählung von dem, was über die Existenz eines Reticulums in der gestreiften Muskelfaser gesagt worden ist, liefert keinen sicheren Grund, weder um es bedingungslos anzunehmen, noch um zu erklären, dass es überhaupt nicht vorhanden sei. Auch die, welche die Präexistenz eines Netzes behauptet haben, stimmen über die Art seines Baus, über die Lage, welche es einnimmt, und über die Genesis der Theile, die es bilden, nicht untereinander überein. Einige halten das Reticulum für interfibrillär, Andere meinen, die Fibrillen seien ein Kunstproduct, und die Faser enthalte während des Lebens einen soliden, geformten Theil, das Reticulum, und einen flüssigen, das Enchylema, das in den Maschen des ersteren enthalten sei; noch Andere nehmen ein sarkoplasmatisches Netz an, das zwischen den Fibrillen oder Fibrillengruppen liege. Es hat nicht an Autoren gefehlt, die das Vorhandensein eines Netzes im Innern der gestreiften Faser entschieden geleugnet und die Bilder, die man von ihm erlangen kann, für Gerinnungszustände erklärt haben, die durch die Reagentien in dem Inhalt der präexistirenden Fibrillen hervorgebracht werden."

coplasm (an entity which they, of course, did not recognize), *but rather the result of staining the entire sarcoplasm and also probably part of the fibrils.* Veratti also extended this criticism to the work of others already mentioned, including Thin, Bremer, and Leydig, stressing that though he did observe reticular structures in chrome-osmium-silver preparations, these structures bore no resemblance to the reticula obtained through gold impregnation, the method upon which most of his predecessors relied.[4]

An examination of these early descriptions substantiates Veratti's criticism. In particular, it should be noted that in the majority of cases where reticula were described, the existence of preexisting fibrils and hence of the sarcoplasm *per se* was not acknowledged, and moreover the disposition of the reticular elements was usually held (Thin, Melland, Marshall, Van Gehuchten, Haswell, etc.) *to be responsible for, not merely spatially related to, the striations.* It is significant that the illustrations accompanying these descriptions (Figs. 1, 3 *a*, 4, 6 to 10) show, as a rule, an interconnected "grid" of longitudinal and transverse elements, related to each other with geometrical precision; the divisions on the vertical and horizontal axes evidently corresponding to the demarcations of the fibrils and sarcomeres respectively. The distribution of the reticula described by Veratti, on the other hand, was of a totally different nature; they clearly lay between and around the fibrils, *within* the sarcoplasm, and although often arranged in phase with the striations, were entirely separate from these; moreover, their arrangement was found to differ characteristically from one muscle to another. On the basis of his definitions, Veratti concludes:

"No one (except Fusari and Cajal) has seen the internal reticular apparatus of the muscle fiber. Only Retzius (in his second memoir) and perhaps MacCallum have obtained images that could be related to the partial coloration of the apparatus itself."

[4] In a personal communication Peachey states that it is clear from an electron microscopic examination of muscle treated by the gold impregnation method employed by Melland (see Fig. 1) that the metal particles are indeed randomly dispersed throughout the entire sarcoplasm and also along the Z bands; an observation in complete agreement with Veratti's supposition.

In his work on insect skeletal muscles published in 1888, Cajal shares the view of Van Gehuchten and others that the fibrils of Kölliker represent coagulated "liquide myosique" (hence noncontractile material), and that the contractile part of the fiber is an orderly series of transverse and longitudinal filaments, thin in the case of leg muscle and thicker in fibrillar flight muscle (Figs. 22 *a* and 22 *b*); exactly, it should be pointed out, as would be expected if, as suggested, the "reticula" described actually represent total impregnation of the interfibrillar sarcoplasm. In the drawing reproduced in Fig. 22 *a* this is especially evident, as in this case a tracheole is shown, embedded in the stained sarcoplasm. In a later paper (1890), however, we find illustrated, probably for the first time, the true intrasarcoplasmic reticulum of Veratti, though Cajal mistakenly supposed that the fine filaments of the system ("capillaires trachéens") were derived from the branching of tracheae either within or on the surface of the fiber (see Figs. 23 to 25). It was again left to Veratti to resolve this confusion between the tracheal and reticular elements in this type of muscle.

An examination of the figures published by Retzius in 1881 shows that he too failed to observe the definitive reticulum in gold impregnated material, although in his second work (1890), in addition to illustrating clearly the distribution of sarcosomes within the fiber, he described (albeit in a fragmentary fashion) a thread-like sarcoplasmic component found in close association with the sarcosomes (Figs. 17 and 18) which probably represents portions of the true reticulum. It is also possible, as Veratti suggests, that MacCallum (1897) observed the reticulum in heart muscle fibers treated with Kolossov's osmium–tannic acid method (Fig. 14); that the membranous edges of the "sarcoplasmic discs" arranged in rosette form around the fibrils actually represent the transverse and longitudinal components of the reticulum, although the reported presence of a transverse "disc" only at the level of "Krause's membrane" (Z band) casts doubt on this interpretation, for, as Veratti showed, the transverse reticulum often straddles the fibril at or near the A-I junction.

Finally, Fusari (1895*a*, 1895*b*, 1895*c*), who was the second, following Cajal (not the first, as Veratti states), to apply Golgi's "black reaction" to striated muscle, described, but did not illus-

trate, series of transverse and longitudinal filamentous reticula corresponding, in general, to those observed by Veratti. He apparently supposed, however, that they represented the interfibrillar sarcoplasm in its entirety.

Briefly, then, the conclusions of Veratti which are of special interest are, firstly, the integrity of the reticulum as a separate component *within* the sarcoplasm, as evidenced especially by the selective staining of the slender filaments of the reticulum within the abundant sarcoplasm of those muscles in which fibrillar material is restricted—for example, certain muscles of *Cyprinus* (Veratti's Figs. 19, 20, and 21) and *Hippocampus* (Veratti's Fig. 23). The second, important observation concerns the orientation of the reticulum in phase with the striations of the fibrils. The third conclusion is that while the spatial relations of the reticular elements (notably the transverse components) may change during the cycle of activity, the elements maintain their integrity at all times and never (as Fusari, for example, supposed) coalesce with one another.

THE IDENTITY OF THE RETICULA DESCRIBED IN STRIATED MUSCLE

We have traced, in a necessarily cursory manner, the history of the identification, up to the start of this century, of reticula situated within the muscle cell. Now the field of study must be widened, to consider the relationship between these structures and reticular apparatuses visualized by means of the same and other techniques in non-muscular cells.

In two short papers (1898*a*, 1898*b*) Golgi, using his "black reaction" method of silver impregnation, described two reticular systems in nerve tissue: a superficial "revêtement réticulaire" in cells of the "spinal marrow" of the cat, and an internal system first in Purkinje cells of the owl *Strix*, and then (1899) in spinal ganglion cells of the horse (Fig. 26). Within a short space of time, the use of this and other methods in the hands of many investigators (*e.g.*, von Bergen, 1904; see Fig. 27) demonstrated endocellular reticula, more or less resembling the classical "apparato reticolare interno" of Golgi, in a wide variety of vertebrate and invertebrate cells. In a review published in 1914, Duesberg listed over two hundred papers dealing specifically with the nature and distribution of this aspect of cytoplasmic organization. In this discussion, the muscle cell was soon fully implicated. Veratti had guardedly concluded that the analogy between the reticula he described and that of other cells was "not such as to authorize us, for the moment, to affirm that the two series of formations are identical," and whereas Retzius had suggested that the reticulum might afford the path by which the nerve impulse is conducted into the fiber, Veratti stated that "there are no data upon which an hypothesis of the functional meaning of the reticular apparatus of the muscle fibers may be based."

One of the most important general hypotheses relating to the function of endocellular reticula was put forward by Holmgren, who, in an extensive series of papers on a wide variety of cells (for references, see Duesberg, 1914), developed the concept of the "Trophospongium," a system of filamentous or canalicular pseudopodia penetrating into the cell body from external trophocytes. This system, which Holmgren considered to have a nutritive function, was envisaged as being changeable in character: now solid, now canalicular and fluid containing, in accord with the metabolic state of the cell.[5] Holmgren's most extensive work (1908) concerned striated muscle, and in this he described a well developed and precisely oriented trophospongial network which corresponds, in general features, to the reticula illustrated by Veratti in similar muscles (Figs. 33 to 38). Holmgren believed his trophospongium to be identical with the internal reticular apparatus of Golgi, though for his part Golgi denied the existence of any connection between the networks he described and the exterior of the cell.

Whereas Veratti was guarded on the comparison between the reticular apparatus of Golgi and the network of muscle, Holmgren supposed that the "Trophospongiennetze" ("binnenzellige Fadennetze") was equivalent to the "apparato reticolare interno" of Golgi, to the "Binnennetze" or "Netzapparat" of Kopsch (1902), and to the "aparato tubuliforme" of Cajal (1904); an equivalence that was in principle frequently accepted, as is implied by the terms "appareil de Golgi-Holmgren" (Sánchez, 1907), "conduits de Golgi-Holmgren" (Cajal, 1908), "Retzius-Holmgrensche Kanälchen" (Maccabruni, 1910), "Golgi-Holmgrensches Netz" (Heidenhain, 1911), and others. But rather than become too deeply involved in

[5] For a synopsis of the numerous preparative methods employed by Holmgren, see Bowen (1928).

questions of terminology, let us briefly examine the evidence upon which these comparisons were made, and for this purpose, the work on reticula of insect fibrillar flight muscle, a tissue that received a good deal of attention, is particularly instructive.

The fibers of fibrillar flight muscle afford a somewhat special case, as Leydig (1859) showed that they are invaded by fine extensions of the tracheal respiratory system, restricted to the surface of the fiber in most other types of insect muscle. The results obtained by Cajal (1890) on application of the silver impregnation technique of Golgi have already been mentioned (Figs. 23 to 25). He noted that fibers so treated contained not only small tracheae (tracheoles), but also a system of fine filaments which were considered by him to be continuous with the tracheoles, and which were "absolutely invisible by all other methods of preparation." These elements, which he termed "capillaires trachéens," formed longitudinal and transverse reticula and are clearly identical with those described by Veratti, who, however, correctly interpreted their nature as being quite distinct from the tracheal system. Fusari (1895*a*, 1895*b*, 1895*c*) apparently ignored the tracheal system altogether; he described reticula in fibrillar muscle, among other types, and equated these with the "primitive unmodified protoplasm of the muscle fiber . . . the sarcoplasm of Rollett."

From the point of view of development of the concept of the sarcoplasmic reticulum, the choice of insect muscle as a favorite object of study by Cajal and later by Sánchez and especially by Holmgren seems, in retrospect, an unfortunate one. The respiratory function of the tracheae (originally suggested by Malpighi) had been repeatedly stressed and demonstrated by investigators in the nineteenth century (for references, see Wigglesworth, 1953), and it had been clearly shown by Leydig (1859) that whereas tracheae and tracheoles are frequently restricted to the surface of the insect muscle fiber, sometimes (as in certain flight muscles) the fiber is deeply penetrated by a complex tracheal system, although this last observation was opposed by Van Gehuchten (1886), Ciaccio (1887), and others.

Both Sánchez (1907)[6] and Holmgren (1908, 1910) gave good illustrations of the definitive reticulum in insect muscle (Figs. 34, 36 to 41), those of Holmgren being especially well executed. Not only did these authors agree, however, that the reticulum carried out a primarily nutritive role, but also both fell into the further error made by Cajal in regarding the reticulum (the "réticulum de Cajal-Fusari des muscles" and the "endozellulären Trachealendnetze," respectively) as continuous with the tracheae. Sánchez was thus misled into believing that an internalized tracheal net was present in all insect muscles, and that:

"Seulement Cajal, à l'aide du chromate d'argent, a pu surprendre l'entrée des trachées dans la matière striée de toutes les variétés musculaires des insectes. Sous ce point de vue, les préparations au chromate d'argent sont tellement démonstratives qu'on s'étonne des doutes de Veratti [*that the reticulum was to be equated with the tracheal system*] et nous sommes portés à croire, que cet auteur n'a peut-être obtenu que des imprégnations imparfaites."

Having accepted without reserve the continuity between the reticulum and the tracheae,[7] Sánchez was committed to accounting for the presence of sarcoplasmic networks in non-tra-

[6] An interesting comparison in detail may be made between the accounts of Veratti and of Sánchez. Veratti, having clearly stated that while certain muscles have *two* transverse reticular elements surrounding each muscle segment (sarcomere), situated at or near the Q zone (A-I junction), others have only one, placed at the level of the Z line, nevertheless goes on to conclude (on the basis of his finding *three* such elements in certain invertebrate muscles) that *all* muscles probably possess the full complement of three: a central component at the level of the Z line, between a pair of Q band components. Sánchez, on the other hand, recognized two entirely separate reticula, both with longitudinal connectives: the first, the "network of Retzius," visualized only by gold chloride methods, having transverse elements at the Z line; and the second, seen only in chrome-silver stained material, placed on either side of this line.

Thus neither author admitted that variation in the positioning of the transverse components of the reticulum was an intrinsic feature of the individual type of muscle under study, a conclusion for which the electron microscope has already afforded ample evidence.

[7] ". . . si cette façon de se produire des trabécules terminales n'implique pas une continuité anatomique et une certaine solidarité fonctionelle avec les trachées, nous ne comprenons pas pourquoi on considère comme étant nerveuses les ramilles finales d'un axone."

cheated fibers, and in constructing such a homology he was forced to refute the well established concept of a gas-filled tracheal respiratory supply in insect muscle:

"Nous ne prétendons pas d'après ce que nous venons d'exposer, que ce réseau terminal issu des trachées forme un appareil tubuliforme plein d'air. Une telle hypothèse est insoutenable depuis que Fusari a confirmé l'existence du réticulum de Cajal chez les vertebrés . . . il serait permis de supposer que les filaments du réticulum renferment un plasma nutritif avec de l'oxigène en dissolution."

Holmgren was even stronger in asserting a homology between the reticula of muscles of insects and of other animals, presumably because the former constituted an undoubted example of the penetration of one cell by another, the chief requirement of his trophospongium hypothesis. He was, however, unable to demonstrate clearly a similar connection between the reticulum and cells (supposedly "trophocytes") lying between the fibers of skeletal muscles of crustaceans and vertebrates. Nevertheless, he assumed an identity between the networks in all types of muscle, and in particular compared the prolongations of the tracheal end cell with the pseudopodia of the cells (possibly actually cells of the endothelium) which he described as lying between, and connecting the sarcolemma with, the blood capillaries in mammalian heart muscle; a comparison that was, as Bowen (1926) stated, "a perilous saving of the homology." Some years before Holmgren's work, in fact, Veratti had clearly shown that there was here no homology to be saved; that essentially similar reticula were present in a wide variety of muscles, regardless of the presence or absence of tracheoles, which he correctly viewed as a special modification; that the reticular system was truly endocellular in nature, and that apparent associations between it and extramuscular elements either were associations of proximity only, or were altogether spurious.

Athanasiu and Dragoiu (1913) also cast doubt on the existence of a reticulum in insect muscle, other than the ramifications of the invading tracheoles. These they described (Fig. 43) in flight muscle fibers of *Hydrophilus* stained by Cajal's reduced silver nitrate method, as constituting a very orderly array in the plane of the "dark disc," from which arose very fine filaments traversing this region of the fibrils and "corresponding to the M-membrane, or stria of Hensen." In leg muscles, however, these authors stated that the tracheoles do not penetrate within the fiber (and correctly related this to the relatively small oxygen needs of this tissue, as compared with wing muscle), but from their failure to find any trace of the reticulum of Veratti in these fibers treated by the methods of Cajal and Golgi, one may infer that in each instance they obtained and described incompletely impregnated material, in which the tracheal system alone was displayed.

The confusion and uncertainty which grew up concerning the earlier descriptions of complex and delicate reticula is well expressed in the statement of Tiegs (1955), who quoted Athanasiu and Dragoiu as having shown a system of tubules "less elaborate than that figured by Holmgren, but corresponding closer to that seen in the living tissue" and, accepting the fact that the tracheal system may be visualized by metallic impregnation, found it "disconcerting that similar networks have been reported by this method in crustacean and even mammalian heart muscle."

At this time, then, it is fair to say that while the literature contained careful accounts of reticular organization in the striated muscle cell, and while the hypothesis of Holmgren in particular (or at least its structural aspects) had a number of adherents, no generally accepted functional concept was available through which the reticulum of muscle and other cells could be related. The nutritive trophospongium visualized by Holmgren thereafter seems rather rapidly to have lost whatever support it at one time enjoyed; indeed, as early as 1910, Bensley wrote:

"The trophospongium theory of Holmgren, as far as I am aware, has found no support. Even if it were admitted for the nerve cells there are many categories of cells in which a reticular apparatus, or a canalicular apparatus is to be found, to which the theory is wholly inapplicable."

Nevertheless, the study commenced by Golgi at the turn of the century continued to flourish, and during the next two decades a wealth of evidence accumulated pointing to the existence of a strictly endocellular component, of almost universal occurrence, which came to be known simply as the "Golgi apparatus" by its proponents, *and the gradual incorporation of the muscle cell into the structural pattern of all cells was effected, almost*

totally, at the expense of the reticula which have so far been considered.

THE SARCOPLASMIC RETICULUM AND THE GOLGI APPARATUS

Just as the early descriptions of reticula in muscle and elsewhere had been defined primarily in terms of the methods employed in their visualization, the history of the Golgi apparatus reflects the history of the development and interpretation of particular techniques of fixation and staining. In a series of six papers, Bowen (1928*a*, *b*, *c*, *d*, *e*, *f*) collected and collated the available methods for demonstrating what was considered to represent the Golgi apparatus in all its manifestations, and he included the methods used by Veratti, Cajal, Holmgren and others to display the reticulum of muscle. However, the "black reaction" of Golgi, which afforded exquisite preparations of the reticulum within the sarcoplasm, was superseded by others judged to be "specific" for the Golgi component.

Soon after the original description of the internal reticular apparatus by Golgi, in 1898, many accounts of apparently similar organelles were published, and these were found to be so widespread that in a review published in 1926 Nath was able to write:

"In the Metazoa the Golgi apparatus seems to be of almost universal occurrence (only the non-nucleated erythrocyte and desquamating epithelial cells being exceptional),"

and by this time it had been discovered in many Protozoa, in addition. Furthermore, a general pattern was observed in the arrangement of this system in different animal groups: while it usually took the form (especially in preparations stained with osmium or silver) of a compact, frequently perinuclear network in vertebrate somatic cells, in vertebrate germ cells and in most invertebrate cells the Golgi apparatus appeared to consist of deeply staining isolated filaments or granules (dictyosomes) either aggregated together or dispersed throughout the cell. While the nutritive function ascribed to the trophospongium by Holmgren was a hypothesis devoid of observational support, with the realization of the widespread distribution of the Golgi apparatus came the gradual implication of this system in the process of secretion. In an extensive and well illustrated review Cajal (1914) described the form and perinuclear disposition (except in nerve cells) of the apparatus in many cell types from the rabbit, cat, and dog (*e.g.*, Fig. 42). Furthermore, he noted marked differences in the appearance of the system in cells at different phases of activity, hypertrophy or fragmentation being correlated with the secretory cycle in, for example, ossifying cartilage and the caliciform cells of the intestine, while in the pancreas the fragmented elements of the Golgi apparatus were found to be closely associated with the zymogen granules as they appeared in the cytoplasm.

A further (and perhaps the best-documented) instance of the secretory role of the Golgi apparatus was demonstrated by work on the formation of the acrosome of the sperm (Gatenby, 1917; and see Nath, 1926, for later references); Nassonov (1923) and Ludford (1925) showed the intimate relation between secretion droplets and Golgi material in glandular epithelia of the salamander and in the epididymis of the rabbit, respectively, and a similar situation was observed in a number of glands by Bowen (1926). Nath (1926) summarized the position as it then appeared:

"It is difficult to deny the secretory function of the Golgi apparatus in gland cells, in certain cases of vitellogenesis and in the formation of the acrosome. We must not, however, hasten to the conclusion that the function of the Golgi apparatus is always secretory, since it is present in non-glandular cells also, such as nerve cells, muscle cells, etc."

It is evident from Nath's account that, in citing the muscle cell, he did not regard the extensive sarcoplasmic network we have so far considered as equivalent to the Golgi apparatus.

Luna (1911) examined mammalian heart muscle treated with silver nitrate after arsenious acid-formalin fixation, and made no mention of an extensive reticular system within the sarcoplasm but instead described localized areas of densely staining twisted filaments lying near the poles of the nucleus (Figs. 47 *a* and 47 *b*) resembling similarly placed structures found in many types of cell,[8] and these he regarded as "equivalent to the

[8] It is possible that the filaments and granules situated in the sarcoplasm around the nucleus which had been noted by Retzius, Fusari, and Veratti actually represented the silver impregnated Golgi apparatus in the sense of the term used by Luna, but these authors appear to have attached little importance to the observation.

'reticular apparatus' revealed by the classical Golgi method in almost all animal cells." Similar observations were made on mammalian smooth muscle (Fig. 46) by Hortega (1913) and a more fragmented perinuclear apparatus was described in muscle of the leech by Sánchez (1913).

Despite this point of correspondence between muscle and other cell types, Beams (1929), in discussing the controversial subject of the Golgi apparatus of muscle, stated:

"It seems to be the general opinion among students of the Golgi apparatus that the so-called Cajal-Fusari network of muscle tissue is homologous with the Golgi material of other tissue. However, it is apparent that the evidence in favor of this conception . . . is not conclusive, although such as exists must be conceded until other evidence is presented."

Beams, using the Mann-Kopsch method, described osmiophilic areas located at the poles of the nucleus in cockroach gut muscle and stressed that these corresponded, *in the same preparations*, to the Golgi apparatus of gut epithelial cells, and he considered that Luna's views though they had been "unpopular" were probably correct. Bowen (1926), on the other hand, questioned the conclusions of Luna and put forward the view, on the basis of admittedly fragmentary impregnation of his material, that the images of muscle reticula obtained by use of the classical Golgi method could be duplicated with the Nassonov-Kolatchev procedure, which he considered "specific" for the Golgi apparatus. He concluded that the "network of Cajal-Fusari represents the Golgi apparatus (and all of it) in striated (skeletal and heart) muscle fibers of vertebrates and invertebrates" (Figs. 44 *a* and 44 *b*). This work, while in no way clarifying the vexed question of the form of the Golgi apparatus of muscle, represents perhaps the last pre–electron microscopic consideration of the reticulum of Veratti and others.

Merland (1934) criticized Beams' work on the ground that the Mann-Kopsch osmium impregnation method that he employed is difficult to interpret, and repeated the investigation with the da Fano technique, which he regarded as highly specific for the Golgi apparatus. He identified as this structure a group of large vacuoles lying in the sarcoplasm near the nucleus—vacuoles which also stained in fresh tissue with neutral red, a characteristic of the "vacuome" of Bensley, Parat, and others. Merland claimed that the Cajal-Fusari network was seen only after prolonged fixation, when the perinuclear apparatus no longer appeared, but the identity of the former system, which was not illustrated, is in some doubt since the author supposed it to be mitochondrial in nature from its response to Regaud's method. Dawson (1929) likewise found neutral red–positive bodies in fresh muscle of *Necturus*, which were also found to be impregnated with silver or osmium by the methods of Cajal and Nassonov-Kolatchev. However, whereas the apparatus of Luna and Merland was positioned solely at the poles of the nucleus, that described by Dawson consisted of "isolated bodies either grouped in longitudinal rows between the columns of myofibrillae, or clustered at the poles of the nuclei." It is to such variation in the results obtained by different workers using the same supposedly specific methods that much of the confusion concerning the nature and distribution of the Golgi apparatus of muscle must be attributed. Macdougald (1936), for example, failed to obtain superposable images of the "vacuome" and the filamentous perinuclear osmiophilic structures in developing muscle of the chick heart (Fig. 45), and concluded that Merland's results were "simply a resurrection of the now discounted 'vacuome hypothesis' [and] must therefore be dismissed."

Eastlick (1937) also demonstrated a similarly localized apparatus in vertebrate skeletal and cardiac fibers, but concluded, after having attempted various permutations of the chrome-osmium-silver method (the black reaction) of Golgi, that the reticula of earlier authors was spurious, and that

"striated muscle does not contain a filamentous Golgi substance with ramifications in the sarcoplasm and . . . the Cajal-Fusari network, assumed to be Golgi substance, is to be explained as an impregnation of the substance of the membranes and bands of the myofibrils per se";

and, failing to distinguish between the results afforded by the silver chromate and gold chloride methods, that

"the various types of 'nets' described [are] due to fixation of fibers in different stages of contraction" (see Figs. 48 and 49).

It is interesting to note that in the accounts mentioned above, the authors hesitate to admit

that the Golgi apparatus and the reticulum might coexist in the muscle cell as separate systems, and this rigidity is well expressed by Eastlick, in his conclusion that

"There is no necessity for homologizing the substances located in the membranes and bands with Golgi material since striated muscle contains osmiophilic bodies which have a position and appearance characteristic of the Golgi substance of other cells. These are the crescents, rods and granules located at the poles, or along the sides of the nuclei of striated muscle."

Because of the uncertain relationship between the reticulum and the Golgi apparatus, the very existence of the former became uncertain, as may be exemplified by the following quotation from Macdougald:

"With regard to the Cajal-Fusari network, which up till 1929 was regarded as representing the Golgi apparatus . . . of striated muscle . . . it is strange that no mention whatsoever is made of it in Luna's paper. Nevertheless, from the works of Cajal, Fusari, Veratti and others, some such structures must be presumed to exist in the fibres of striated muscle, but that this is the Golgi apparatus (for cardiac at least, but also most probably for voluntary muscle) must be denied."

Finally, in this survey, Sosa and Menegazzi (1940) may be mentioned, primarily because they, of all those concerned with sarcoplasmic organization, were among the very few who distinguished clearly between, and accepted the independent existence of, a general reticulum and a restricted perinuclear system in the muscle cell. In large measure, however, the correspondence of the latter component to the Golgi material of other cells was established at the expense of the delicate and highly organized intrasarcoplasmic reticulum, which thus became, until the advent of thin-sectioning techniques for electron microscopy, a virtually forgotten component of muscle, or rather a component which was generally considered to be spurious.

The world into which the Golgi apparatus of the muscle cell was thus introduced was not, however, a restful one. While the study of the reticulum languished after the early part of the century, the controversy on the Golgi apparatus grew and flourished. Although, as has been mentioned, good evidence was obtained implicating this system (at least in some cells) in the process of secretion, the terminological difficulties which had originally centered around the reticulum were later matched and surpassed in the case of the Golgi apparatus.[9] As Kirkman and Severinghaus (1938*b*) wrote:

". . . many mistakes have arisen, not from erroneous theorizing, but from lack of specific tests for different cytoplasmic components, also from regarding such non-specific tests as osmic acid and neutral red as specific."

The application of thin sectioning for electron microscopy to studies of cell structure perhaps came, as it were, just in time to save the Golgi apparatus from further tribulation, as Palade and Claude (1949*a*, 1949*b*) suggested that the system was a gross artifact having no corresponding morphological counterpart in the living cell but rather representing myelin figures, formed during fixation from phospholipid material. Shortly afterward, Dalton (1952, 1953) described a system of threads, granules, and droplets in the cytoplasm at the poles of the nuclei in liver and intestinal cells of the mouse, concluding on the basis of these observations (which were later to be confirmed and extended, as improvements in the preparation of material were formulated) that "the classical form of the Golgi substance in fixed material is a rather close approximation to its form in living cells."

With the rapid advances in specimen preparation that followed, many of the technical difficulties that had hampered light microscopists in their attempts to integrate and compare the structure of striated muscle with that of other cells were obviated, and the reinstatement of both the Golgi apparatus and the sarcoplasmic reticulum, as distinct structural entities, was made possible. The method of silver impregnation employed by Veratti displayed the variable character of the sarcoplasmic reticulum in different types of muscle. The procedures of electron microscopy have confirmed the existence of this variation, and have provided a structural and topographical analysis of the components of the reticulum. From such information, together with that gained from

[9] See reviews by Cajal (1914), Cowdry (1924), Nath (1926), Bowen (1929), Kirkman and Severinghaus (1938*a*, 1938*b*, 1938*c*), Hirsch (1939), Hibbard (1945), Bensley (1951), Palay (1958), etc., for considerations of this problem.

corresponding physiological and biochemical studies, is emerging an increasingly clearer picture of the relation between form and function in the striated muscle cell.

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FIGURE 1

Melland (1885) employed the method of gold impregnation in his investigation of striated muscle, and his interpretation of the structure of this tissue, which he believed contained a reticular framework and a fluid matrix, is embodied in his diagrams reproduced here. The so-called transverse elements represent the Z band of the myofibrils, which Melland believed did not exist in the living fiber; the longitudinal connectives probably represent optical sections of the grossly stained interfibrillar sarcoplasm.

Diagrams 1 and 2 show three-dimensional aspects of the network postulated by Melland, and in Diagram 3 is depicted a fiber in longitudinal section, the transverse net (actually the Z band) being described as a "row of dots crossing the fiber in the position of Krause's membrane." The H bands of the fiber were thought to be "minute thickenings of the longitudinal bars of the network midway between the transverse networks."

FIGURE 2

A figure from the work of Bremer (1882) in which he developed the hypothesis that the muscle fiber is composed of separate "corpuscles" (actually the scattered nuclei), each of which was thought to be produced into a protoplasmic reticulum of "muscle rods." This diagram represents a single corpuscle with a portion of its network, which was visualized as bearing alternately thick and thin transverse filaments (the Z and M bands of modern terminology), linked by longitudinal connectives.

FIGURES 3 *a*, 3 *b*, *and* 4

Marshall (1888) shared the view of Thanhoffer, Gerlach, and others that the nerves reaching the fiber connect with the filaments of the postulated reticulum, which were visualized as stemming from discrete "muscle corpusles" (Fig. 4). These corpuscles in fact represent the fiber nuclei, and the supposed derivation of transverse trabeculae from them is shown in Fig. 3 *b* (Marshall, 1890), while in Fig. 3 *a* the longitudinal connecting elements are included; the striation of the fiber was thus attributed to the precise geometrical alignment of these two arrays.

FIGURE 5

A diagram from Carnoy (1884), illustrating his concept of muscle structure: that the muscle cell differs from others primarily in the degree of order shown by its protoplasmic "reticulum." In this case, Carnoy imagined that he had observed the transformation of cells of the gut of *Hydrophilus* into muscle, and the bands occurring where the "reticulum" became oriented into a grid of cross-meshes (*my*) were thought to represent regions where the "myosin" was concentrated in the "enchylema" and was deposited in the form of granules. The "reticular hypothesis" of protoplasmic organization put forward by Carnoy was supported and elaborated by Van Gehuchten and his followers in their work on the structure of striated muscle.

FIGURES 6 TO 10

Van Gehuchten, following Carnoy, was the chief proponent of the "fluid matrix hypothesis" of muscle structure. He believed that the fibrils were the coagulation products of a "myosin-rich" fluid which, in the living fiber, surrounded a reticular array of more solid elements, considered to be the contractile portion. His views are illustrated in the figures here reproduced from his first memoir (1886). His ideas initially received much attention, and figures from the work of several of his contemporaries who supported his views are shown elsewhere on this plate.

Fig. 6. Part of a fiber from the crayfish *Astacus* showing the precisely organized reticulum thought to remain after extraction of the fluid matix.

Fig. 7. Illustrates Van Gehuchten's concept of the appearance of a living fiber from the beetle *Melolontha*. The transverse reticulum (Z band) is evident, also the longitudinal connectives in the A band region, the optical properties and density of which were attributed to localized concentration of the matrix. The rows of dots in the clear region (I band), which may represent sarcosomes, were termed "accessory discs" by Van Gehuchten and were believed to be local thickenings in the longitudinal trabeculae, which disappeared on contraction (see Fig. 8 *b*).

Fig. 8 *a*. A fiber corresponding to the last, after fixation. The coagulated fluid matrix was supposedly deposited on the meshes of the reticulum in the "dark band" (A band), where the filaments were therefore thicker.

Fig. 8 *b*. A fiber from the larva of *Melolontha*, showing a "fixed contraction wave." This figure illustrates Van Gehuchten's concept of contraction as a shortening and thickening of the longitudinal links of the reticulum, resulting in the appearance of dark contraction bands.

Fig. 9. Illustrates a portion of a fiber of *Noctuela* after alcohol fixation. Van Gehuchten described numerous variations in structural detail of the reticulum; in this instance the M band is represented by a dense dot at the center of each longitudinal trabecula (compare with Fig. 8 *a*).

Fig. 10. Representing a transverse section through a group of alcohol-fixed fibers of *Melolontha* muscle, showing their component myofibrils. Van Gehuchten admitted the existence of such fibrils in the living fiber only in the case of "dissociable" flight muscles of certain insects.

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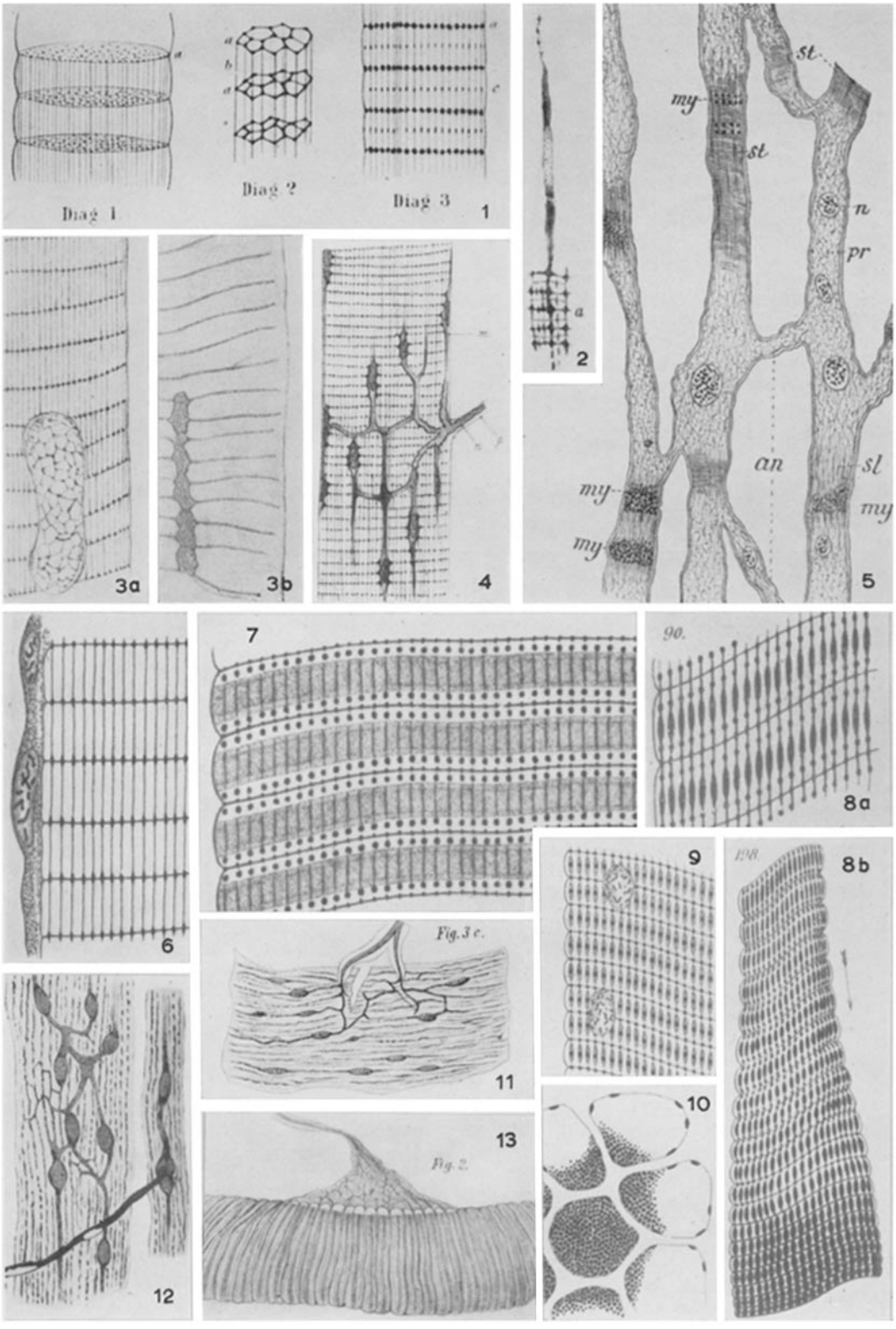
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FIGURE 11

An illustration from Thanhoffer (1882) of a nerve ending in gold impregnated frog muscle. He believed that in this tissue, the nerve was subdivided into longitudinal filaments which connected with nuclei lying beneath the sarcolemma rather than with an internal reticulum as was believed to be the case in insect muscle.

FIGURE 12

Diagram of gold impregnated frog muscle from Gerlach (1877), who believed, with Marshall, Bremer, and others, that the fiber is composed of separate bodies or cells associated with a reticulum, which connect directly (as in this figure) with nerve branches at the end plate.

FIGURE 13

Here, in a fiber from the beetle *Hydrophilus*, the end plate was described by Thanhoffer as containing a network, the elements of which apparently passed into Krause's lines (Z bands).

The reticula described and illustrated in this plate do not correspond in any way to the definitive sarcoplasmic reticulum of Fusari, Cajal, Veratti, and others, but were the product of a basic misconception of muscle structure, in which the striations of the fibrils and the interstices between them were mistaken for transverse and longitudinal filaments, embedded in a homogeneous matrix.

FIGURE 14

MacCallum (1897) described the sarcoplasm of human and other heart muscle as being divided into a series of chambers defined by radial and transverse membranes, arranged around the fibrils in a rosette pattern as in the diagram reproduced here. Veratti considered it possible that what MacCallum believed to be sections through the membranes of sarcoplasmic discs or chambers were in reality filamentous elements of the reticulum, though from the fixation MacCallum employed this seems unlikely.

FIGURES 15 AND 16

Retzius (1881) denied the existence of fibrillar organization in striated muscle, and believed that the fiber contained a skeleton of filaments embedded in an optically homogeneous matrix. He conceived of these filaments as outgrowths of muscle "cells" (Fig. 15, gold preparation of *Turdus* muscle) which, though themselves probably not contractile, were considered to be responsible for the conduction of the nerve impulse into the fiber. The rows of nodules or thickenings seen in Fig. 15 and more clearly in Fig. 16 (similarly prepared *Dytiscus* muscle) were described as transverse profiles of parallel rows of the muscle cell filaments, which became superimposed when the section was oblique, as in the left of Fig. 16, to give the appearance of cross-striation. It is apparent that the thicker "filaments" (first order net) represent the Z band, while the intermediate striation (the H band) seen in the upper part of the figure was accounted for in the same way, as superimposed images of a system of "second order filaments."

This geometrically precise reticular model proposed by Retzius, based primarily upon Golgi-impregnated material, is similar to that of Melland, Marshall, Bremer, Van Gehuchten, and other authors who likewise believed that the matrix of the fiber was fluid and who, having rejected the view of fibrillar organization, were obliged to relate the phenomenon of striation to the orderly arrangement of the postulated filaments of the reticula.

In a later paper (1890), Retzius put forward a radically altered view, admitting the existence of fibrils within the living fiber, and probably became the first author to describe elements of the definitive sarcoplasmic reticulum (Figs. 17 and 18).

FIGURES 17 AND 18

In his second work on striated muscle (1890), Retzius abandoned his earlier views and accepted the opinion of Kölliker and others that the living fiber contains parallel arrays of fibrils which are themselves cross-striated and contractile. He further recognized the existence of an interstitial "serous fluid" between the fibrils ("Zwischensubstanz" of Kölliker, "Sarcoplasma" of Rollett), within which he observed varying numbers of granules "suspended on longitudinally arranged filaments" as seen in Figs. 17 and 18, showing chrome-osmium treated muscle of the beetle *Oryctes* and of the hagfish *Myxine* respectively. He correctly identified these granules as "sarcosomes," noting, for example, that they are unusually abundant in insect flight muscle, while the fine filaments probably represent tubules of the true sarcoplasmic reticulum. Retzius described this last system incompletely however, making no mention of transverse connectives, and it appears that a more complete impregnation of this system is obtained by Golgi's "black reaction," employed by Fusari, Veratti, and others.

In this work, Retzius stated that the supposed reticula described in gold–acetic acid treated fibers by himself (1881) and by other authors represented

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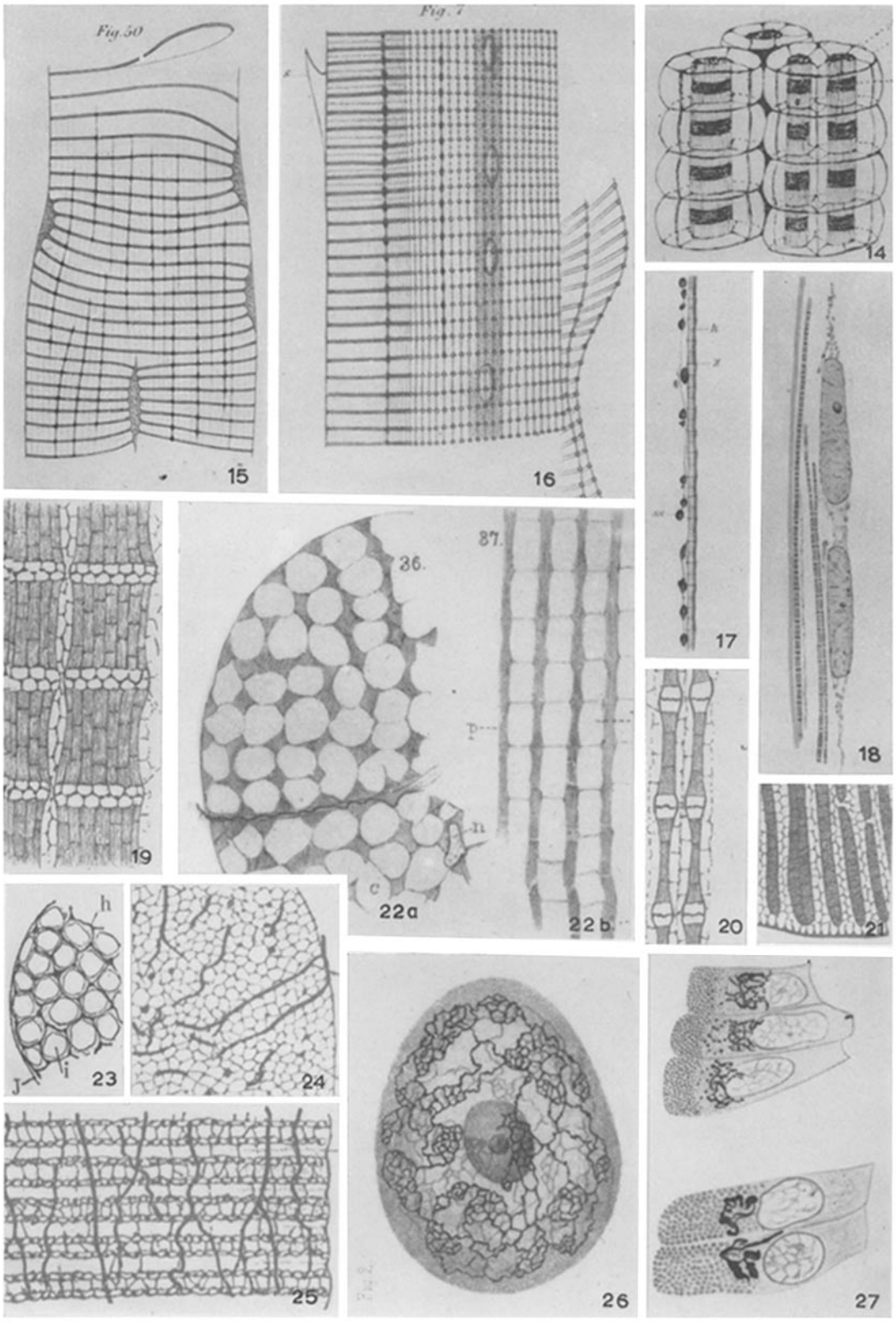
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merely the interfibrillar sarcoplasm, reduced in extent by the swelling of the fibrils induced by the fixation and staining procedure; a view that is here considered to be essentially correct.

FIGURES 19 TO 21

Diagrams from the work of Bütschli and Schewiakoff (1891), representing the structure of muscle from the centipede *Scolopendra*. They believed that the sarcoplasm of the fixed fiber contained a honeycomb of filaments, similar in longitudinal and transverse sections (Figs. 19 and 21), and distinct from another series of reticula which was thought to traverse the light (I) band and attach together adjacent A regions. The Z band (Fig. 20) was believed to represent the latter reticulum, seen in tangential longitudinal section.

It is probable that the reticula described by these authors represent an artifact produced by fixation (in alcoholic picric acid), and they bear little resemblance to the sarcoplasmic reticulum later described by Veratti and others.

FIGURES 22 *a* AND 22 *b*

Rollett and Veratti contended that the muscle reticula described by many authors in material impregnated with gold chloride did not represent differentiations within the sarcoplasm, but were the product of generalized coloration of the entire interfibrillar material, and examination of the figures and descriptions of Cajal (1888) such as those reproduced here, of gold stained flight muscle of *Hydrophilus*, supports this belief. In the figure of the transverse section, the deeply stained polygonal areas (of the supposed reticulum) clearly represent the whole sarcoplasm, and in one place a trachea is shown, traversing this. In the longitudinal section, the "transverse elements" probably represent the Z bands of the fibrils. In a later paper, however, Cajal (1890) undoubtedly described the definitive reticulum of the insect muscle fiber, although he misinterpreted its nature.

FIGURES 23 TO 25

Illustrations from the work of Cajal (1890) on insect muscle prepared by the Golgi silver impregnation method, in which he described, for the first time, a system of filaments forming a reticulum lying within the sarcoplasm between the myofibrils. This system is clearly shown in Fig. 23, depicting sections of *Ateucus* muscle, and these should be compared with Cajal's earlier (1888) paper, in which he incorrectly interpreted the entire sarcoplasm as constituting a reticulum, in material impregnated with gold. In leg muscle of *Acridium* (Fig. 25), he described a more complex situation, in which paired transverse elements straddle the fibrils on each side of the Z band; in *Acridium* flight muscle he observed a reticulum associated with the tracheae penetrating the fiber.

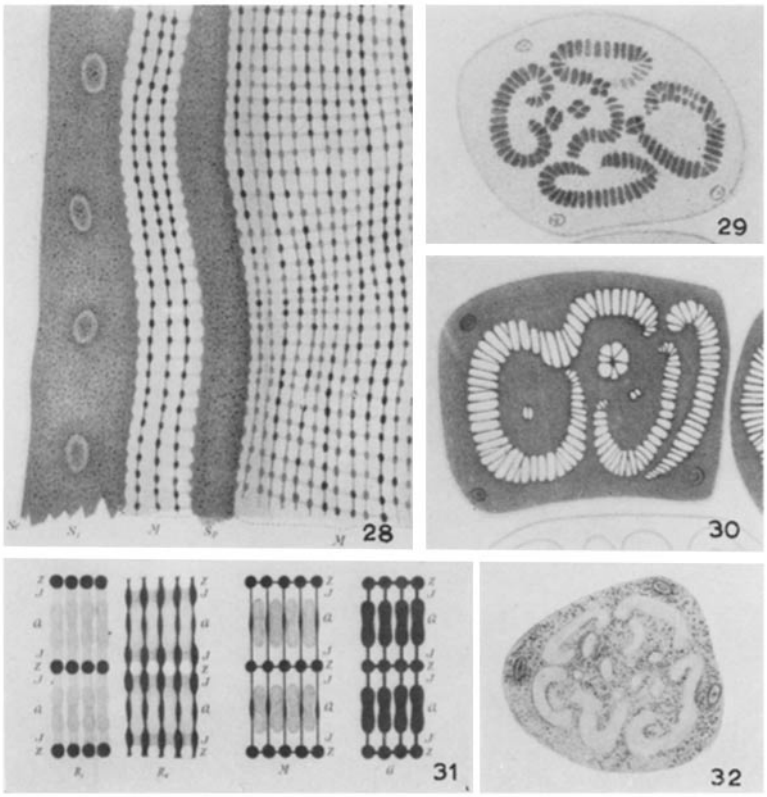
Cajal misinterpreted the nature of the reticula he observed, however, and believed that they represented the ultimate branches of the tracheal system an error made subsequently by Sánchez and Holmgren.

FIGURE 26

A figure from the work of Golgi (1899) illustrating the silver impregnated "endocellular reticular apparatus" in a spinal ganglion cell of the horse. This system was originally described by Golgi (1898*a*) in Purkinje cells of the owl *Strix* and was found to be present in nerve cells of other animals examined (1898*b*, 1899). The more localized bodies subsequently visualized by this and similar impregnation methods in virtually all cell types investigated came to be generally regarded as homologous elements of the "Golgi apparatus."

FIGURE 27

Von Bergen (1904) described localized networks of the Golgi apparatus in a wide variety of cells. The figures reproduced here show the appearance of this system in epithelial cells of the dog prostate. The positioning of the filaments beneath one pole of the nucleus was found to be characteristic of many cell types.



FIGURES 28 TO 32

The work of Rollett (1888) is of great importance in the history of the development of concepts of muscle organization, as he not only introduced the term "sarcoplasm," but was the first to criticize objectively, on the basis of his definition of the sarcoplasm, the views of several of his contemporaries who had described so-called reticular systems within the muscle fiber. Rollett showed conclusively that these descriptions related to the impregnation of the sarcoplasm in its entirety, and left open the question of the existence of true reticular elements which, he said, might prove to be present *within* the sarcoplasm, and which were soon to be discovered and described by Fusari, Cajal (1890), Veratti, and others.

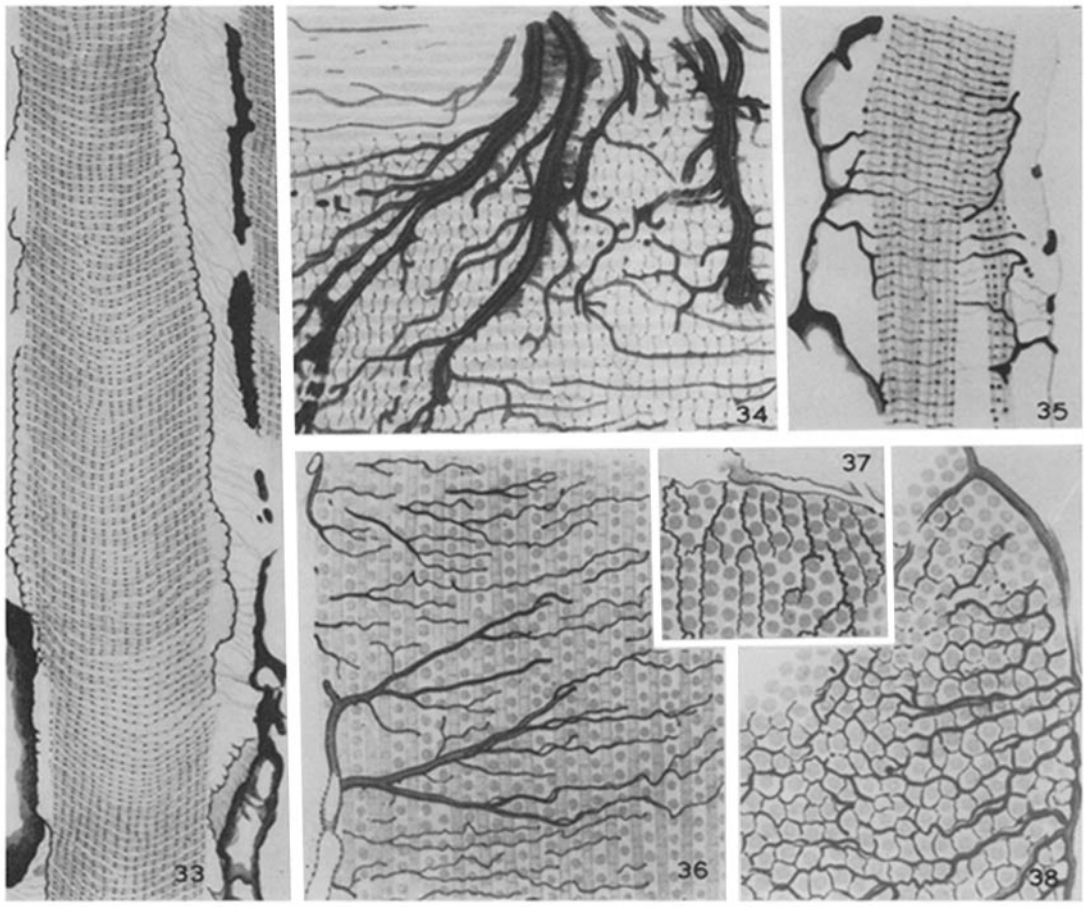
The figures from Rollett's work reproduced here illustrate well the observational background for his suppositions, for he found in certain muscles of *Hippocampus* fibers in which the fibrillar material was very restricted in extent and hence in which the sarcoplasm (in his sense) was abundant. In fibers fixed in alcohol and stained in hematoxylin (Fig. 29), only the groups of fibrils (fields of Cohnheim) and not the sarcoplasmic matrix were colored. However, in gold chloride stained fibers (Fig. 30), a "negative" image was obtained; only the surrounding sarcoplasm was darkly stained, while the fibrils were colorless, and in longitudinal sections of fibers thus treated (Fig. 28) the dark sarcoplasm between the fibrils was considered to be responsible for the often reported appearance of a reticular formation. In fibers treated with osmium, Rollett found that, as in the case of gold chloride, the fibrils were not affected, and that coloration of the sarcoplasm was progressive (Fig. 32).

Fig. 31 summarizes his views. The first image represents the appearance of four cross-striated fibrils at a low-focus position; the second, showing a reversal of light and dark areas, at a higher focus. The third and fourth represent the models of Melland and of Van Gehuchten respectively, and these he relates to the variation in the appearance of his fibrillar model under different optical conditions.

FIGURES 33 TO 38

A group of figures from the work of Holmgren (1908) illustrating the sarcoplasmic reticulum of striated muscle, stained with the chrome-silver "black reaction" of Golgi or by Holmgren's trichloracetic-resorcinol-fuchsin "trophospongium" method (Fig. 38).

Fig. 33. Longitudinal section of muscle from the mouse diaphragm. Holmgren believed that fine filaments (supposedly nutritive channels) traversed the sarcolemma and connected the fiber with the intensely stained blood capillaries, a situation that he considered homologous with the penetration of tracheoles into insect muscle—both examples being offered in support of his



general hypothesis of the nutritive "trophospongium." The system of fine filaments he described in this muscle represents transverse components of the sarcoplasmic reticulum lying on each side of the Z band, near the A-I junction—an arrangement that has been observed in several muscles with the electron microscope—but the longitudinal elements of the reticulum described by Veratti do not appear to have been observed.

Fig. 34. Longitudinal section of flight muscle of the fly *Asilus*, in which the internalized tracheal system is stained, together with the fine filaments of the sarcoplasmic reticulum, believed by Holmgren to be continuous with the tracheoles ("terminale Trachealnetze"), but which have been shown in the electron microscope (Smith, 1961) to represent tubular processes derived from the plasma membrane sheath drawn into the fiber with the tracheoles.

Fig. 35. Longitudinal section of heart muscle of the crayfish *Astacus*. In this instance, Holmgren described a thick "trophospongial net" traversing the Z band, and another parallel system at the level of the Q band. The thickness of the sarcolemma may be attributed to the heavy deposition of silver dichromate which occurs during this staining treatment.

Fig. 36. Longitudinal section of *Bombus* flight muscle, in which the silver salt was deposited only on the main (extramuscular) tracheal trunks and on the penetrating tracheolar branches, leaving the reticulum unstained. Note the fibrils, and the sarcosomes lying between them in the sarcoplasm. A corresponding transverse section is shown in Fig. 37, to be compared with Fig. 38.

Fig. 38. Transverse section of flight muscle of the bee *Bombus*. This figure may be compared with that of Veratti (1902, Pl. 4, Fig. 38), which depicts a similar muscle, but in which the correct proportions of, and hence the distinction between, the reticulum and the tracheoles is more clearly indicated. Note that the filaments of the reticulum in both figures are seen to run in the sarcoplasm between the fibrils.

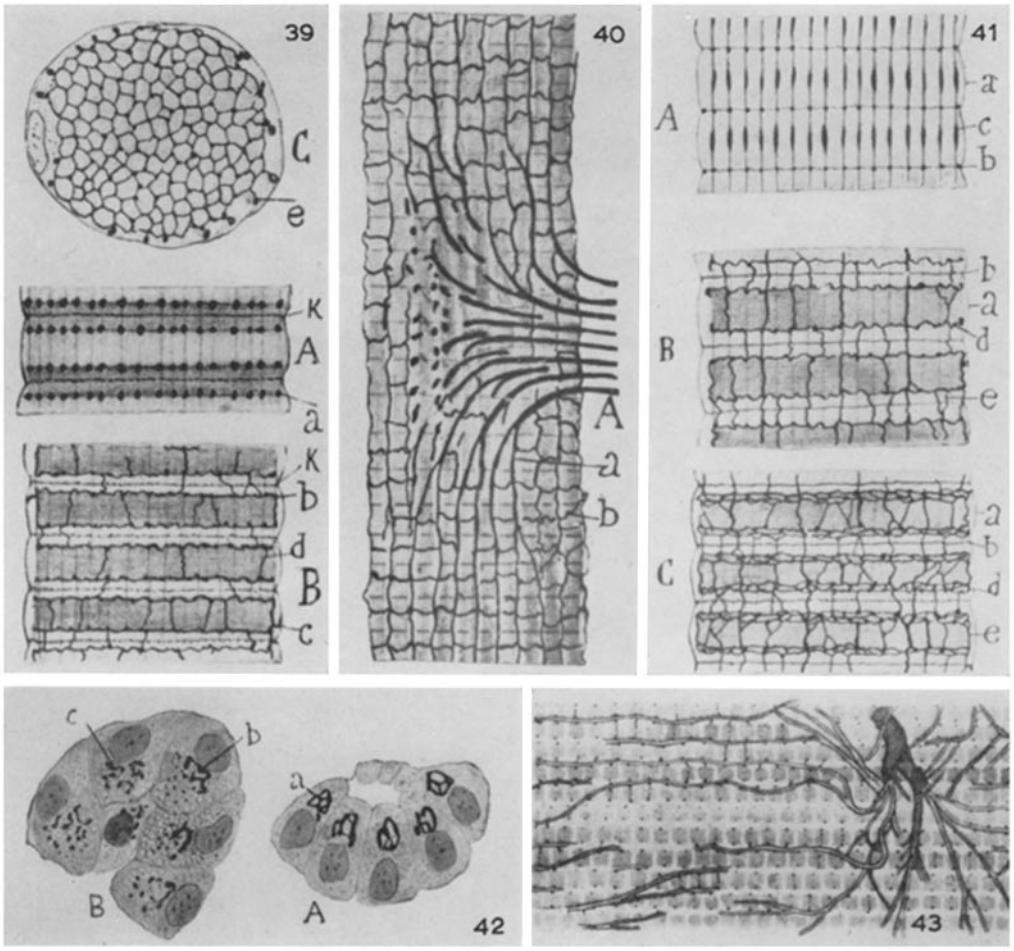
FIGURES 39 TO 41

Illustrations from the work of Sánchez (1907) on the reticular apparatus of striated muscle.

Fig. 39. Preparations of muscle of the cat, stained by the silver dichromate method. *B* represents the transverse reticulum placed on each side of the Z band (line of Krause) at the A-I junction of modern terminology, linked by longitudinal elements. The appearance of the reticula in transverse sections of the fiber is shown in *C*. The image depicted in *A*, in which only the Z band and a double row of granules (possibly sarcosomes) were stained, was considered atypical.

Fig. 40. Showing a longitudinal section of flight muscle of *Hydrophilus*, in which the ramifying tracheal system is seen penetrating within the fiber (*a*), together with a fine network (*b*) which Sánchez, like Cajal, mistakenly thought was continuous with the tracheae, but which Veratti correctly recognized as an entirely separate formation within the sarcoplasm.

Fig. 41. *A* represents a section of leg mucsle of *Dytiscus* impregnated with gold, in which, Sánchez considered, reticula were visualized that were quite separate from those seen in silver preparations. The former, composed of elements situated at the Z level linked by longitudinal connectives, was that recognized by Veratti as the product of impregnation of the Z bands of the



fibrils and of the interfibrillar sarcoplasm, rather than a distinct structure *within* the sarcoplasm. *B* and *C* represent silver impregnated images of leg muscle of the beetle *Geotrupes* and of an unspecified insect respectively. It is clear, from a comparison between these figures and those of Veratti, that Sánchez described the definitive sarcoplasmic reticulum, but he mistakenly supposed that even in insect skeletal muscle the reticulum was continuous with the tracheal system.

Sánchez never admitted the existence of a silver stained transverse reticular component at the level of the Z band, whereas Veratti, despite his accurate descriptions of the variation in disposition of the reticular elements in different muscle, considered that "complete" impregnation would always be found to reveal *three* transverse reticula (one at the Z band level and others on each side of this) and attributed the variability he observed to differential staining, and not to intrinsic morphological features.

FIGURE 42

Two figures from the extensive work by Cajal (1914) on the Golgi apparatus. Cajal described deeply staining bodies situated near the nucleus in a wide variety of cells, and in the instance included here illustrated, in material impregnated by the uranium nitrate–formol method, the disruption of the Golgi apparatus associated with the appearance of zymogen granules, in secretory cells of the rabbit pancreas.

FIGURE 43

Athanasiu and Dragoiu (1913) examined flight muscle of the water beetle *Hydrophilus*, treated by the reduced silver nitrate method. They described only the branching system of tracheoles running between the fibrils, and did not detect the delicate reticulum associated with this invading tracheolar system.

FIGURES 44 *a* AND 44 *b*

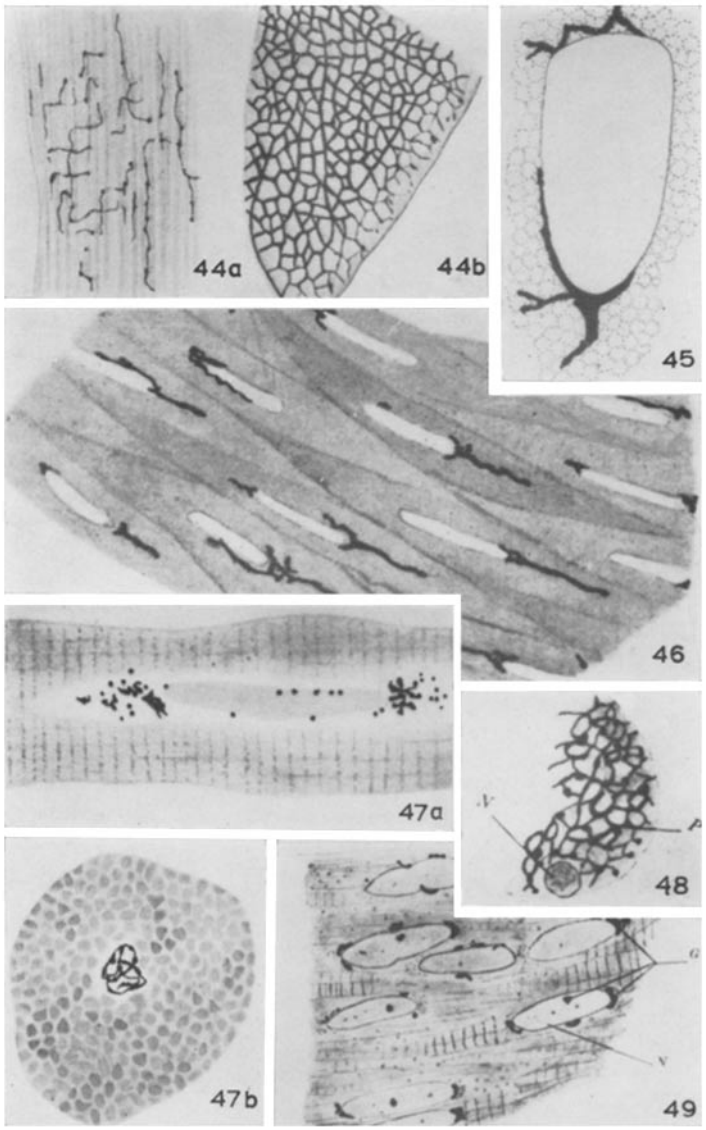
Two figures from Bowen (1926) of muscle of the cat, impregnated with osmium by the Nassonov Kolatchev method. In Fig. 44 *b*, the entire sarcoplasm has been stained, while in Fig. 44 *a*, only fragments of the Cajal-Fusari network are visualized. Bowen believed that the latter represented the Golgi apparatus of the muscle cell, though Luna and others had shown that deeply staining bodies were present near the nuclei of muscle, resembling what were considered to be Golgi elements in a wide variety of cell types.

FIGURE 45

Macdougald (1936) found that the form of the Golgi apparatus in embryonic chick heart muscle was essentially similar to that described by Luna. This figure is of a 10-day embryo, in which the filaments were impregnated with silver by means of the da Fano technique.

FIGURE 46

An illustration from the work of Hortega (1913) showing a thread-like Golgi apparatus impreg-



nated by Cajal's reduced silver nitrate method, situated near the poles of the nuclei in smooth muscle cells of the dog bladder, and closely resembling that described by Luna (Figs. 47*a* and 47*b*) and others in striated muscle fibers.

FIGURES 47 *a* AND 47 *b*

Two figures from the work of Luna (1911), illustrating the disposition of the deeply staining filaments lying in the sarcoplasm near the poles of the nucleus in heart muscle of the rabbit prepared by Golgi's arsenious acid method. The observations of Luna gave the first clear indications that the form of the Golgi apparatus of muscle was similar to that in many other types of cell.

FIGURES 48 AND 49

Two figures from the work of Eastlick (1937) on striated muscle of vertebrates. Fig. 49 depicts deeply staining bodies visualized at the poles of the nucleus, lying in the sarcoplasm, in turtle heart muscle impregnated with osmium by the Kolatchev method. These structures, resembling those described by Luna, Macdougald, and others, were correctly designated by Eastlick as the Golgi material of the fiber. However, in preparations such as that shown in Fig. 48 from Kolatchev impregnated frog skeletal muscle, Eastlick concluded that the networks of Veratti and others were artifacts, produced by the impregnation of part of the fibrils as well as of the entire sarcoplasm.