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Biological Significance of the Early Structural Alterations in Skeletal Muscle Fibers Infected by *Trichinella spiralis*

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Biologische Bedeutung struktureller Skeletmuskelveränderungen nach Infektion mit Trichinella spiralis

Zusammenfassung. Es wurden Trichinose-befallene Skeletmuskelfasern von Ratten elektronenmikroskopisch untersucht. Die durch die Larven erzeugten schweren Veränderungen in der Faser-Ultrastruktur manifestieren sich in folgenden Befunden:

1. Vermehrung der sarkoplasmatischen Matrix.
2. Zersplitterung und Lyse der Myofilamente.
3. Hyperplasie des RS-Systems.
4. Hyperplasie der T-System-Tubuli mit Bildung von eigenartigen multi-tubulären und multi-vesiculären Komplexen.
5. Hyperplasie und Hypertrophie der Mitochondrien.
6. Vermehrung der Golgi-Komplexe.
7. Vergrößerung und Vermehrung der Kerne.

Diese Veränderungen können als Ausdruck der spezifischen Anpassung der durch parasitäre Abbauprodukte stimulierten Muskelzellstruktur gelten. Sie sind weder Zeichen einer Degeneration noch cellulärer Abwehrvorgänge („Parenchymatöse Entzündung").

Summary. Skeletal muscle fibers of the rat were studied with the electron microscope during the first stages of *Trichinella spiralis* infection. Severe changes in the fine structure caused by the larvae within the muscle fiber may be summarized as follows: 1) Increase of the sarcoplasmic matrix. 2) Fragmentation and lysis of the myofilaments. 3) Hyperplasia of the RS system. 4) Hyperplasia of the T system tubules which develop peculiar multitubular and multivesicular complexes. 5) Mitochondrial hyperplasia and hypertrophy. 6) Increase in number of the Golgi complexes. 7) Enlargement of and increase in number of the nuclei.

Such changes can be explained as a specific adaptation of the muscle cell structures stimulated by the parasite metabolites. They are not degenerative phenomena or defensive cellular processes ("parenchymatous inflammation").

Introduction

One of the most striking features about the trichinous infection is the preference shown by the emigrant *Tr. spiralis larvae* to invade fibers of the voluntary muscular system of some mammals (Virchow, 1860; Hemmert-Halswick and Bugge, 1934; Niño, 1934; Jensen and Roth, 1938; Gould, 1945).

Although these emigrant *Tr. spiralis larvae* have been found in multiple organs and tissues (Frothingham, 1906; Mauss and Otto, 1942), it is widely acknowledged that only in the striated muscle fibers do they find the suitable medium for their subsequent development into infective forms (Romanowitch, 1912). In other locations (brain, liver, pancreas, retina, and even in the myo-

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cardium) the larvae coming from the blood capillaries are rapidly surrounded by a cellular inflammatory exudate which invariably destroys them (FROTHINGHAM, 1906; GOULD, 1945; RIBAS-MUJAL, 1950).

Description based on studies made with the light microscope indicate the muscle fibers invaded undergo structural and histochemical modifications immediately after penetration of the larvae. Fundamentally these modifications are tumefaction of the sarcoplasm, numerical and volumetric increase of the nuclei (FIEDLER, 1864), vacuolar degeneration of the fiber, desintegration of the myofibrilar striations, more or less extensive segmentary necrosis (EHRHARDT, 1896; GRAHAM, 1897) and changes in the enzymatic activity (BULLOCK, 1953; HANKES and STONER, 1958). Consequently, the sarcoplasm gradually becomes a homogeneous and basophilic mass (known as "Nevinny's basophilic halus") which surrounds the parasite and forms the inner layer of the cyst (NEVINNY, 1927). On the whole, it has been thought that these modifications are degenerative phenomena taking place in the infected muscular fiber (GOULD, 1945; LARSH, 1963). Nevertheless, FASSKE and THEMANN (1961), when studying with the electron microscope the changes in the various structures of infected skeletal muscle fibers, found evident structural alterations in the latter. They considered such changes to be the expression of the metabolic sarcoplasmic activity rather than of a degenerative nature. They based this hypothesis on observation of hyperplasia both of mitochondriae and smooth sarcoplasmatic membranous systems, as well as of the nuclear hyperplasia by amitosis, which can be observed only in invaded fibers; this accentuation of muscle cell metabolism was, in their opinion, a defense mechanism of the fiber against the noxious action of the parasite. They thought, consequently, that these structural changes were an instructive example of the so-called "parenchymatous inflammation" in the presence of a living noxa.

In the present work, the initial changes in the structure of infected muscle fiber are described with the aim of elucidating, in so far as it is possible, their morphogenesis and their biological signification. We shall try to determine whether they are degenerative phenomena, whether they are defensive cellular phenomena in the sense suggested by FASSKE and THEMANN, or whether, on the contrary, they must be considered as a specific accommodation of the muscular structures with new metabolic conditions imposed by the parasite and its metabolites inside the sarcoplasm (STONER and HANKES, 1955).

We deal with the changes that take place from the time the parasite enters the muscle fiber to the complete disappearance of the myofilaments, a phenomenon taking place approximately 48 hours after the penetration of the larva into the fiber. We think it is essential to point out here that it is difficult to establish a chronological correlation of muscular changes in the trichinous infection, since the Trichinella larvae do not invade all the fibers simultaneously. They reach the muscle cells one by one as they are released rhythmically by the adult females localized in the intestines during the period of invasion which lasts several weeks. Therefore, when studying the skeletal muscles in the rather advanced stages of this period of muscular invasion, one may observe, alongside some well developed larvae, other smaller ones that have just penetrated into neighbouring muscle fibers.

The changes corresponding to the more advanced stages of infection will be described in another paper dealing with the genesis of the trichinous cyst.

Materials and Methods

White rats of both sexes weighing 150 to 200 g were infected by feeding them meat containing 500 ± 25 cysts of *Trichinella spiralis*. For studying this early phase of the infection, rats were killed with ether anesthesia 8, 15, and 21 days after they ingested the trichinous meat.

Fragments of lingual, intercostal and diaphragm muscles were fixed in glutaraldehyd and cut into small blocks which were then postfixed in osmium tetroxide in phosphate buffer with sucrose. Embedding was made in Durcupan ACM (Araldit, Fluka). Sections were obtained with a Porther-Blum MT 1 microtome. Thin sections were stained with either lead citrate, uranyl acetate or both together. They were examined under an electron microscope Elmiskop I (Siemens).

Results

The emigrant trichinella larvae originating in the intestines reach the skeletal musculature by the blood stream. As it is rare to find them inside the capillaries (RIVERA-POMAR and RIBAS-MUJAL, 1967) or inside the connective endomysial tissue. We believe the worm must go rapidly through the wall of the capillaries and penetrate immediately a fiber. Usually the fiber harbours only one parasite located more or less axially inside a cavity of circular transversal section, the volume of which is slightly greater than that of the worm. Besides the larva, this cavity contains a matter permeable to electrons in which float granular and membranous sarcoplasmic debris.

No changes in the structure of the muscular fiber are evident immediately after the penetration of the worm, except in the sarcoplasmic zone sourrounding the parasite cavity. The latter is delimited by a sarcoplasm, with ill-defined edges, in which like fringes, debris of RS membranes and isolated granules of ribonucleins and glycogen appear. The parasite cavity never has a delimiting membrane of its own.

The first noticeable changes appearing in the infected muscle fiber develop not only near the worm, but also in areas far away from it, probably extending over the entire length of the fiber. These changes consist of: a) increase of the sarcoplasmic matrix, b) fragmentation and focal loss of the myofilaments. c) hyperplasia of the RS tubules and the T system, d) various modifications of the other structures of the muscle cell (mitochondriae, Golgi complexes, glycogen granulations, ribosomes, nuclei and nucleoli).

Increase of the Sarcoplasmic Matrix

It is a gradual process, but one which reaches great intensity. It causes not only a distinct separation of the myofilaments (Fig. 1), but also a marked enlargement of the perinuclear fields. This phenomenon produces the enlargement of all the fiber, specially around the parasite. On the whole, the density of this matrix is lower than that of the neighbouring non-infected fibers.

Changes in the Myofilaments

The ordered arrangement of the different bands of the sarcomere is lost very early on in fibers affected by the presence of the worm. In contrast, this arrangement is preserved in the neighbouring non-infected fibers. This disorder is synchronic with the already described increase of the sarcoplasm and with the initial

Fig. 1. Longitudinal section of an infected skeletal muscle cell illustrating the early changes induced by *Tr. spiralis larva*. Zigzagging Z-lines (*Z*), increase of the sarcoplasmic matrix (*O*), longitudinal arrangement of the triads (*x*), hyperplasia of the RS tubular system, formation of multitubular complexes (*TC*) of the T system, and disarray of the mitochondriae can be observed. × 20,500

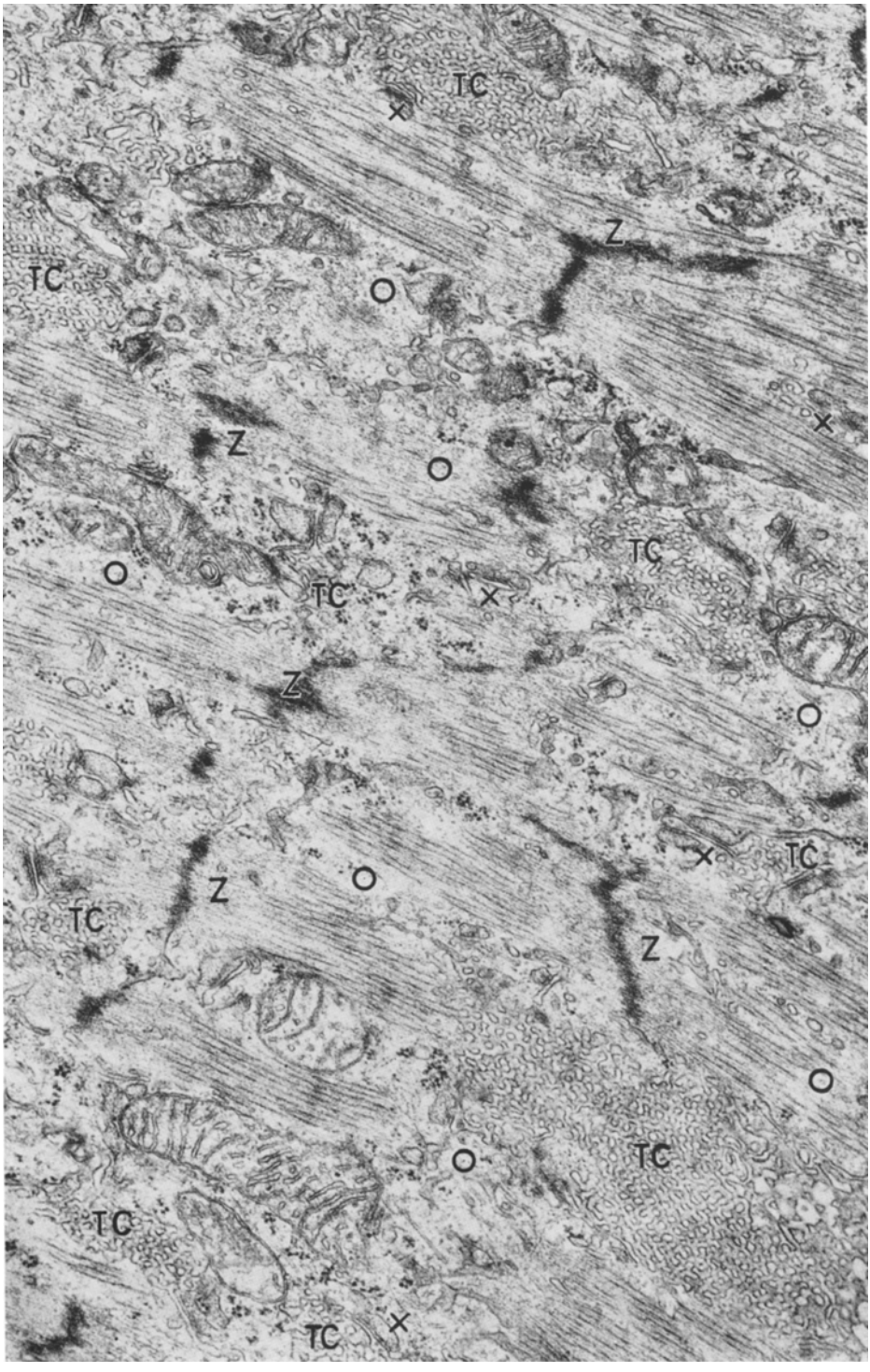

Fig. 1

changes of the RS tubular system and with T system. The Z bands become discontinuous, zigzagging and irregular in thickness (Fig. 1). This phenomenon may be subsequent to the progressive increase of the sarcoplasmic matrix which favours changes of orientation and the shifting of the myofilaments. Nevertheless it is possible that they may be isolated contractures of some of the myofilaments. This fact is difficult to establish because the A and I bands of the sarcomere appear very imprecisely at this moment.

The loss of the contractile elements occurs in a very irregular fashion. Beside sarcoplasmic zones practically devoid of myofilaments, they appear others in which the dissolution is just beginning, and others which preserve their normal structure (Fig. 1). Desintegration seems to affect the various proteic macromolecular components of the sarcomere (myosin, actin and Z-band material) in a very arbitrary manner. We have not found any relationship between these foci of lysis and the constant presence of some cytoplasmic organoid.

Changes in the Tubules of the RS-System and in the T-System

Simultaneously with the development of the hyperplasia of the sarcoplasmic matrix and with the myofibrilar lysis, there starts an intense proliferation of the tubules of RS-system and of the T-system.

The identification of these two systems is usually easy. The RS tubules and cisternae are limited by a membrane of low density whose outlines are somewhat imprecise. The T tubules have a dense membrane and clear outlines. The contents of the cavity of the T-system are generally permeable to electrons. The membrane of both systems present the typical three-layered structure of the unit membrane.

The initial changes can be observed in the triads. Their three tubules change their characteristic transverse orientation for a longitudinal or oblique one, without losing their position on either side of the Z-band and on a level with the A and I bands. This phenomenon is sometimes accompanied by a slight dilatation of one or both RS terminal cisternae. Less frequently, we find in other triads that the lumen of the central tube of the T system is enlarged.

Immediately, a diffuse proliferation of the various RS tubular segments (intermediate, longitudinal and fenestrated collars) starts along the whole length of the fiber, both in intermyofibrilar spaces and in perinuclear fields.

In short stretches of this network some ribosomes are attached to the outer surface of the RS tubules. Therefore, beside long stretches with a smooth surface, they appear now short granular segments. Usually, this hyperplasia is more intense in the perinuclear fields than in the intermyofibrilar spaces.

As in normal fibers, the RS system opens out on to the perinuclear space. We have found no openings of this system on the surface of the fiber. Simultaneously with this diffuse development of the RS system, tubules of the T system of the triads, start to grow both on a level with the A bands and in the subsarcolemmatic spaces, and also in some perinuclear areas. The openings of the T system on to the fiber surface are very obvious (Fig. 2). On the other hand, we have found no continuity between the T tubules and the perinuclear space. The relation between the T system and the Golgi complexes is one of mere proximity.

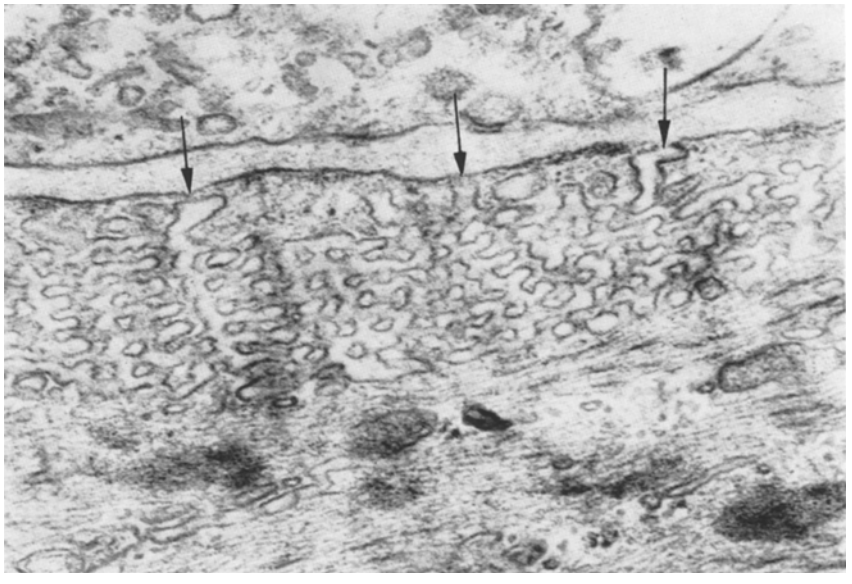

Fig. 2. A subsarcolemmatic OMC showing some openings out on to the cellular surface (↙). × 55,000

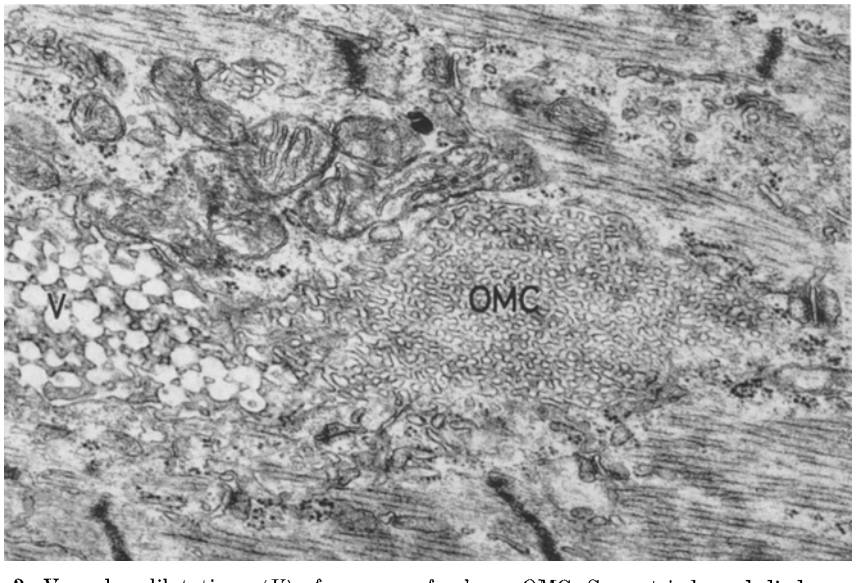

Fig. 3. Vacuolar dilatations (*V*) of an area of a large OMC. Some triads and diads can be seen beside the multitubular complex. × 25,000

In all stages which are about to be described, it is possible to find contacts of the T tubules with the RS terminal cisternae, enlarged or otherwise, forming triads or diads (Figs. 1 and 3).

The proliferation of the T system is not diffuse, but focal, and it does not affect all the triads. Consequently, tight tubular networks ("initial multitubular

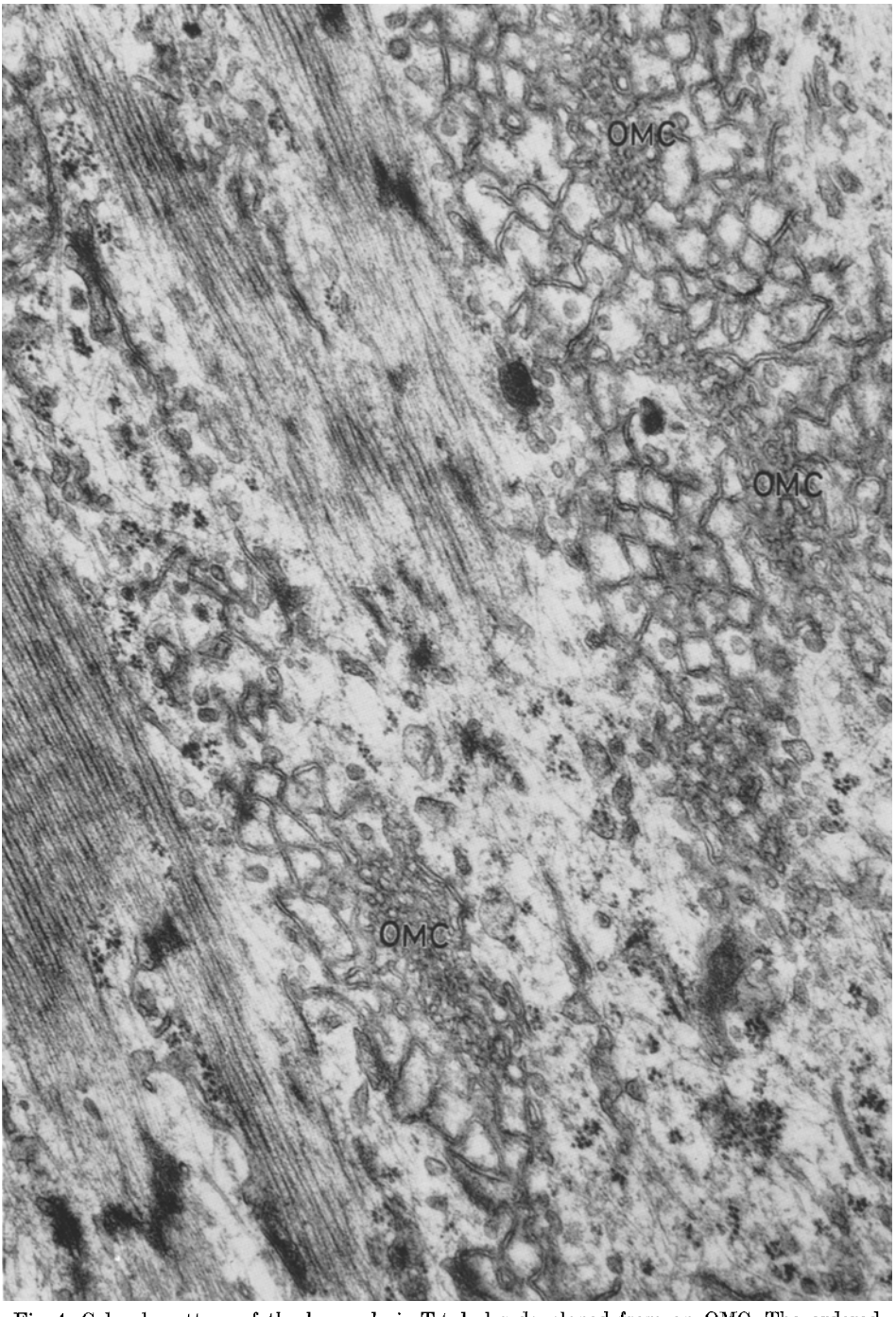

Fig. 4. Cobweb pattern of the hyperplasic T tubules developed from an OMC. The ordered arrangement of the tubular network can still be found in the center of the cobwebs. × 32,500

complexes", IMC) are formed very rapidly and these make up a small ovoid complex whose greater axis is generally longitudinal. These IMC appear all along the fiber no matter where the larva is situated (Fig. 1).

The arrangement of the tubules in these complexes is completely irregular, but they soon adopt a very orderly structure forming tridimensional labyrinths (Figs. 2 and 3) which we shall call "ordered multitubular complexes" (OMC). These OMC are formed by a tridimensional network of anastomosed tubules at right angles. As these tubules are equidistant one from another, and as their diameter is very uniform (350 Å), the complex is very regular. Schematically the axis of the tubules form a tridimensional quadrille ruling which recalls the crystalline structure of the sodium chloride.

The contents of the tubular lumen are permeable to electrons. Between the meshes of the OMC there is a sarcoplasmic matrix, slightly denser than that of the rest of the sarcoplasm, which is devoid of glycogen granulations and ribosomes.

The tubules of a OMC do not intertwine with those of the neighbouring complexes; neither do so with those of the RS.

These multitubular structures slowly reach a stage of great development, without losing their special internal conformation (Figs. 1 and 3). Their outline is always ovoid, and their main axis takes the orientation of the axis of the fiber. In some cases, their greatest diameter exceeds the length of three sarcomeres.

In this stage, the subsarcolemmatic OMC are connected with the cellular surface by means of tubules which often show sacciform dilatations (Fig. 2). These tubules and dilatations sometimes contain a rather dense homogeneous material in continuity with a substance of similar appearance deposited between the plasmolemma and the basal membrane of the altered fiber. This substance will later on form part of the wall of the trichinous cyst. Not all the OMC undergo the same process of development even within the same fiber. Often, vacuolar dilatations appear in the more peripheral tubules (Fig. 3) whilst the central ones remain unchanged. This dilatation is usually not very marked; generally, they reach about 130 millimicrons in diameter. Occassionally, one or more flattened cisternae encircle the complex forming only one peripheral layer. On the other hand, it can often be observed that around, and starting from the OMC, there develops a network of anastomosed tubules which form wide and irregular meshes. The pattern formed by these meshes, arranged in a radial sense ardound the OMC recalls a cobweb (Fig. 4). At this point the tubules of these networks occassionally intertwine with the RS elements. A few mitochondriae and glycogen granules can also be found near them.

In a later phase all the tubules and vacuoles become flattened cisternae of small width, which are interanastomosed one with another. The most peripheral of these arrange themselves in a stratified and concentric form, whilst the central ones group themselves in sorts of whirlpools around secondary centers. In this way "multivesicular complexes" (MVC) are constituted; these occupy extensive areas and their greatest diameter sometimes exceeds 15 microns (Fig. 5).

Many alveoly of small diameter (800 Å) protrude from the external part of the vesicular membranes; they communicate with the lumen of the latter by means of small circular openings. The outline of the membranes is less precise and

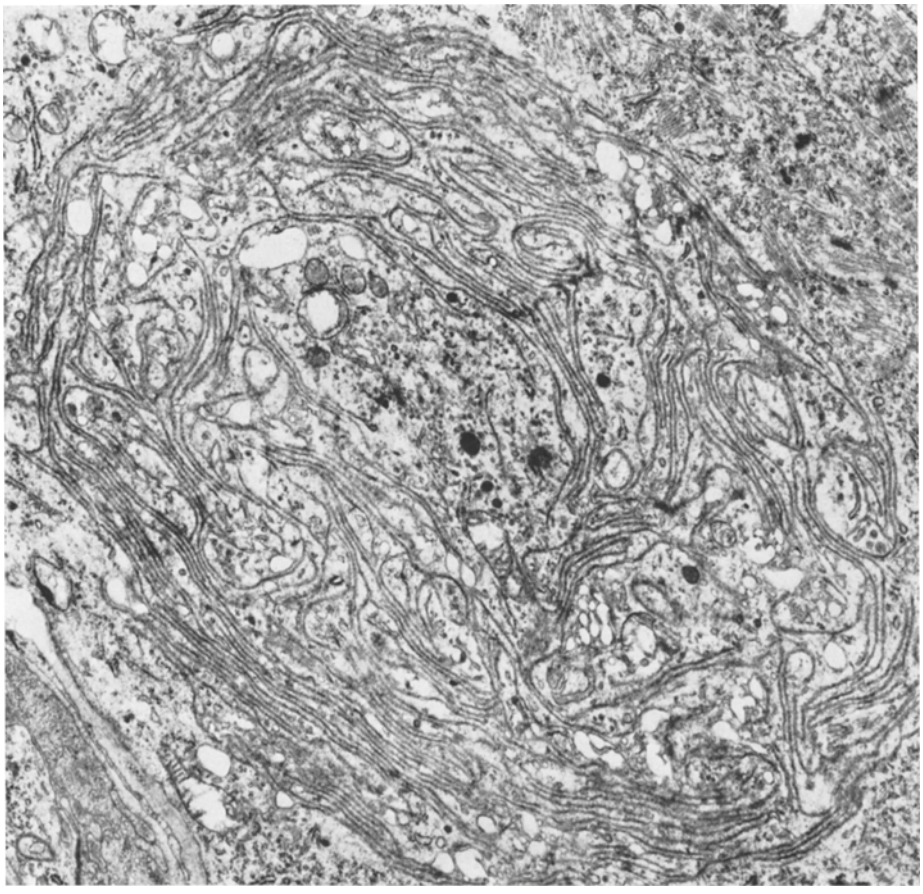

Fig. 5. Flattened cisternae of small width arranged in a concentric form are developed from the cobweb pattern of the OMC thus giving rise to multivesicular complexes. The cisternae are interanastomosed one with another. × 12,500

thinner in the alveoli than in the vesicles. This gives the impression that the alveolus is distended (Fig. 6). A restricted vesicular dilatation can be found on the edges of the cisternae. The contents of the cavity of the flattened vesicles and of the alveoli is permeable to electrons. Exceptionally we have found dilated vesicles containing a fine granular and homogeneously dense material.

The membranes of the MVC are very close to each other, and therefore there is little sarcoplasmic matrix inside the complex. We did not find ribosomes attached to the external wall of the vesicles during any stage of its development. On the other hand, in later stages, glycogen granules, RS tubules of smooth and sometimes granular surface, are often found (Figs. 5 and 6). Some small mitochondriae can also be observed.

The RS tubules and the T system communicate with the central parasite cavity through ruptures in their membrane.

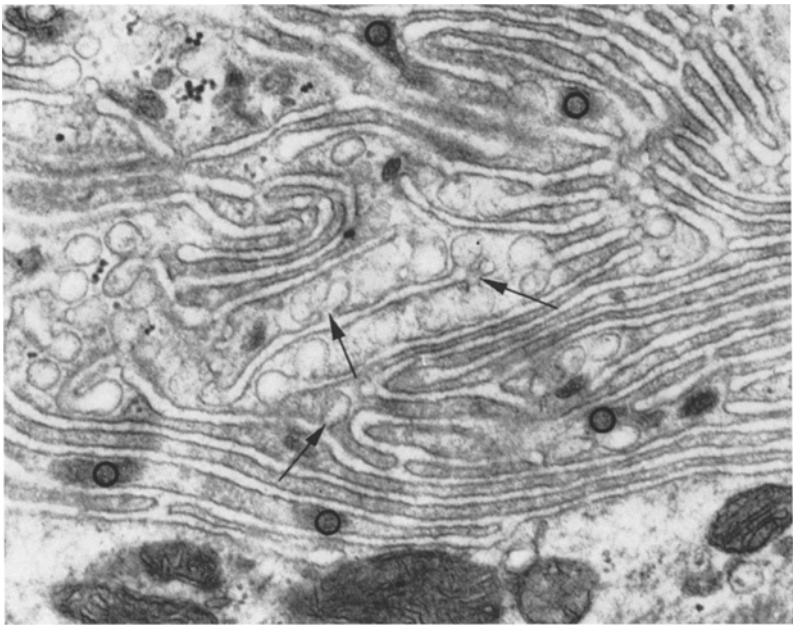

Fig. 6. Alveoli of small diameter bud from the external surface of the vesicular membranes of the MVC. Its lumen communicates with the cavity of the flattened cisternae by means of small circular openings (↙). The alveolar membrane of the cisternae. Some RS tubules (*o*) can be found among the cisternae. × 36,000

Mitochondriae

They lose their usual orderly arrangement in relation to the Z bands and place themselves between the myofilaments on a level different to that of the sarcomere; later on, once the MVC have been formed, they remain outside these, in areas where there are many RS tubules and Golgi complexes. The density of the mitochondriae also increases in the perinuclear fields. This fact is due both to hyperplasia and to a moderate hypertrophy of the mitochondria.

No great changes can be observed in their structure at this stage. Just a few osmiophilic granulations appear in the matrix of some of them. Some mitochondriae with longitudinal cristae can also be found sometimes.

Golgi Complexes and Centrioles

The Golgi complexes, which are scarce in normal muscle fibers and which are grouped at nuclear poles, also seem to increase in number as the rest of the cytoplasmic elements develop. The situation of these complexes continues to be essentially perinuclear. As for their structure, as the larva develops, they seem to be constituted by a great number of flattened, dilated cisternae containing a material permeable to electrons; these cisternae are surrounded by a great number of vesicles of 400 Å in diameter. Some of these are full of a dense material. Numerous microtubules can sometimes be found near the Golgi complexes.

Only in a invaded fiber, have we been able to find one centriole near the Golgi complexes in a paranuclear position.

Glycogen Granulations

They disappear in early phases and reappear later on, as the complexity of the MVC increases. Great amounts of granular glycogen can be observed in some infected fibers.

Ribosomes

One of the first signs of sarcoplasmic changes is the increase of the ribosomes, which appear either isolated or grouped in poliribosomes. As the RS system develops, an increasingly greater number of ribosomes become attached to the external surface of the cisternae. They never adhere to the membranous wall of the vesicles or tubules of the T system.

Nuclei and Nucleoli

A peculiar phenomenon of the first stages of trichinous infection is the displacement of the nuclei towards the axial zone of the fiber on a level with the larva and near the central parasite cavity (EHRHARDT, 1896). This nuclear displacement which like the hyperplasia and hypertrophy has already been described by means of the optic microscope, can also be observed with the aid of the electron microscope. The nuclei, increased in volume, now present numerous ribonuclein granules condensed in the proximity of the nuclear membranes, sometimes forming dense clusters. In the central part of the nucleus the chromatin density is low, and therefore, the nucleus have a vesicular aspect. In some nuclei, we have been able to distinguish structured inclusion bodies as well as elongated bodies of fibrillar or crystalloid nature.

The nucleolus show a great development, specially in the early stages of the cytoplasmic changes. It is formed, for the most part, by a granular material with broad spaces of nucleoplasm. Clusters of chromatin granules of low density, associated with the nucleolar reticulum, can often be found. Fibrillar material is scarce and formed by small spherules.

Discussion

Observations with the electron microscope evidence the situation of the developing larva inside the muscular fiber (Fig. 1), as VIRCHOW pointed out in 1848. We have never found developing larvae in the connective endomysial tissue, as CHATIN (1883) and some parasitologists (BRUMPT, 1936; GARNHAM, 1960; MAEGRAITH, 1966) pretended. Dead larvae surrounded by inflammatory resorption granulomata can be found only sporadiacally within the endomysium (RIBAS-MUJAL, 1950).

DALTON and BONGERT'S (1932) hypothesis of the intravascular development of the worms must also be rejected. Larvae are only present in the blood vessels during emigration from the intestines to the other viscerae of the host, and their presence there is very fleeting (RIVERA-POMAR and RIBAS-MUJAL, 1967).

This marked preference of *Trichinella spiralis* larva for such a specific cellular type, — the skeletal muscle cell — can be understood because only in such a situation does the worm find substances (immediate principles, mineral salts,

water, enzymes) and conditions (oxygen pressure, redox potential, isolation, etc.) necessary for its normal development (LARSH, 1963; ROGERS, 1965).

The parasite finds its "external medium" in the liquid which bathes it inside the parasite cavity (Fig. 1). This fluid contain the basic products for its energetic needs and the synthesis of its own tissues during its development. At least some of these products result from the gradual lysis of the sarcoplasm, as can be seen from the fringe-like edges which delimit the cavity. According to HANKES and STONER (1958) this lysis must be related to the high aminopeptic activity of the worm. Through this mechanism the various normal compounds of the sarcoplasmic matrix (myohemoglobin, glycogen, enzymes, mineral salts, etc.), lipid inclusions, ARN and proteins released by the desintegration of the membranes (RS and T systems), and, at this initial stage of infection, the products of myofibrilar lysis would pass into the liquid of the parasite cavity. Products of synthesis also appear in the liquid, due to the activity of the hypertrophied membranous systems; among them there are enzymes like the alkaline phosphatases, very abundant in the sarcoplasm of the infected fiber (BULLOCK, 1935).

Products resulting from the parasite metabolic activity, can also be found in the liquid of the cavity. According to HASKINS and WEINSTEIN (1957) these are composed of ammoniac and amino-acids (leucine, phenilalanine, alanine, valine, proline, glutamic acid, glycine, serine and methionine) and also of various amines, generally considered toxic to the host (methyl-, ethyl-, propyl-, butyl-, and heptyl-amine, cadaverine, ethanolamine and 1-amino-2-propanol).

It is commonly acknowledged that some of these products cause the symptoms of the muscular invasion period of trichinosis. We also think that these substances change the homeostasis of the infected skeletal muscle cells; so, they may be responsible for the structural changes that the fiber undergoes. It is possible they are diffused through the sarcoplasmic matrix, but, at the same time, the possible role played by the various tubular and vesicular complexes of the RS and T systems must not be forgotten, since their hyperplasia must have a functional signification.

It is interesting to observe how the T type tubules and vesicles form an extensive labyrinthic network which opens out, both on to the external space between the plasma membrane and the basal muscular membrane and on to the parasite cavity, by means of ruptures caused by the process of sarcolysis already described. The possibility therefore exists that substances coming from the exterior of the fiber may penetrate into the parasite cavity, or vice-versa, through successive systems of cisternae (OMC, MVC) which perhaps represent coordinated enzyme chains.

It has been emphasized (RIBAS-MUJAL, 1950) that there is no inflammatory reaction (edema, leucocytic infiltration) around the greater part of the altered fibers and that when this reaction takes place it is usually of slight importance. This fact seems to suggest that the muscular fibers are generally capable of neutralizing the toxic effects of the larval metabolites. The absence of inflammatory infiltration seems to indicate that this neutralization is complete. On the contrary, inflammatory reaction, followed or not by reabsorption granulomata, would indicate an inability to complete the full degradation of the larva metabolites.

These metabolic phenomena of neutralization must occur in the sarcoplasmic matrix, in the lumen of the membranous tubular and vesicular systems or in both. The labyrinthic complexity of the MVC guarantee a prolonged period of contact of the parasite metabolites with the sarcoplasmic matrix, prior to its shifting to the exterior of the fiber. It is also possible that, at this stage, there may occur some immunologic phenomena peculiar to trichinosis since the larva segregates products with antigenic activity (OLIVER-GONZALEZ, 1940).

While the system of T tubules offers the possibility of a direct relationship between the parasite cavity and the surface of the fiber, the whole ensemble of tubules derived from the RS system suggests the existence of a direct communication between the parasite cavity and the perinuclear space. Really, the RS tubules communicate with the parasite cavity by means of ruptures caused by the sarcoplasmic lysis of the wall of the cavity, and furthermore, openings of these tubules on to the perinuclear space can be often observed. On the other hand, as in normal fibers, we have found no communication between the RS tubules and the exterior of the cell, although there are a great number of such tubules in the subsarcolemmatic zone. This direct parasite cavity-perinuclear space relationship may facilitate the passage of the metabolites from the parasite to the nucleus. This fact is important if we try to explain the structural changes of the infected muscle cell by the genetic control mechanisms which normally preserve the cellular homeostasis. Of course, it is possible that the arrival of the new products in the nucleus stimulates phenomena of enzymatic induction and repression, responsible for the formation of new enzymes. These, by synthesis or molecular degradations, would accommodate the cellular structure to the new situation created by the penetration of the parasite.

The nuclear changes in these early phases of trichinosis (shifting of the nuclei towards the parasite cavity, their hypertrophy and hyperplasia, the enlargement and reticulation of the nucleolus) (FIEDLER, 1864; EHRHARDT, 1896; GRAHAM, 1897) seem to imply an active participation of the nucleus in the creation of the new homeostasis of the trichinous fiber. The balance of the metabolic host-parasite relationship is thus guaranteed. The situation of ribosomes attached to some membranous segments also speaks of the existence of active protein synthesis there.

In our opinion, the fibrillar and sarcoplasmic changes by *Tr. spiralis* can not be compared to the degenerative muscular processes caused by freezing (PRICE, HOWES and BLUMBERG, 1964) or drugs (PRICE, PEASE and PEARSON, 1962). On the contrary, as FASSKE and THEMANN (1961) suggest, we think they indicate an increased metabolism. If the lysis of the myofilaments could hypothetically have a degenerative signification, it is just as probable that this could be a mere phenomenon of structural re-organization. The hyperplasia of the T and RS systems, the nuclear changes, the development of the Golgi complexes, the modifications of the mitochondriae are phenomena that do not seem to have a regressive signification. Nor do we think that they are a characteristic exemple of the so called "parenchimatous inflammation", a concept which we reject. On the contrary ,we suggest that they can be explained as a re-structuration of the muscle cells caused by the metabolites of the worm.

Faced with the new internal physico-chemical conditions created by the presence of the parasite, the skeletal muscle fiber can lose its typical and specialized contractile differentiation and adquire structural characteristics of a metabolic type, useful both to the host (desintoxication and immunological phenomena) and to the parasite (creation of a suitable "external medium") (ROGERS, 1965).

Although, as was already known by clasic microscopy, some muscular fibers degenerate at different stages of the infection and cause the formation of a resorption granuloma, usually, most of infected muscular fibers adapt themselves to the new conditions by a process of re-differentiation.

Experimental studies we are carrying out on the regeneration in distal segments of invaded fibers once the poles of the cyst had been completely closed, show that this new structural condition of the trichinous fiber is reversible too, in its turn. In view of this, we think that the sarcoplasmic changes of the muscular fibers invaded by *Tr. spiralis larvae* are not a mere re-differentiation, but a typical example of biological modulation.

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