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Trichinosis

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Trichinosis is a worldwide zoonotic disease closely related to cultural and dietary habits caused by a nematode *Trichinella* spp. Human infection is acquired through ingestion of undercooked meat containing infective encysted larvae. There are two cycles of transmission, one domestic and the other wild. A complete life cycle develops in a single host harboring adult worms in the small intestine, from which newborn larvae migrate and finally encyst in striated muscle. Traumatic and immunological alterations are responsible for the main clinical features, including diarrhea, febrile syndrome, myalgias, oculopalpebral signs and eosinophilia. Cardiovascular, lung and CNS involvement characterize severe trichinosis. CNS inflammatory infiltration and damage may result from larval migration and vascular obstruction, or from the effect of toxic parasite antigens, or eosinophil infiltration. Humoral and cellular immune host response are relevant both to protect against re-infection and for immunodiagnosis. DNA probes and PCR technology may help to identify *Trichinella* spp. Muscle biopsy may disclose *T spiralis* larvae coiled within a muscle fibre host nurse cell surrounded by a capsule. Inflammatory infiltration includes monocytes, plasma cells, eosinophils and T lymphocytes mainly of the suppressor/cytotoxic phenotype. Histological appearance and histochemical profile of the host nurse cell differ from that of striated muscle fibre and are partly indicative of regeneration. Our own histological and histochemical findings in experimental studies of infected mouse muscle support the concept that changes induced by the larva encysting within a single host skeletal muscle fibre which becomes a nurse cell are unique of *Trichinella* infection. Interestingly, no dystrophin could be detected within the host nurse cell-capsule interface. It has been advanced that larva-induced host muscle fibre changes may be regulated at muscle gene transcription level whilst host regulatory pathways governed by cell cycle phase may also contribute to larval development.

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Introduction

Trichinosis, also known as trichinellosis, is a parasitic disease present in almost all regions of the world and produced by a nematode. Human infection, acquired mainly through the ingestion of undercooked pork and its products harboring the encysted larva, is characterized by a febrile syndrome, with myalgias, oculopalpebral signs, diarrhea and eosinophilia and leading eventually to death.

The disease exists since very ancient times, and the Old Testament (Lev.11: 4,7) quotes an admonition against eating swine: "...And the swine, though he divide the hoof and be clovenfooted, yet he chewed not the cud; he is unclean to you."

Epidemics have been recorded since 427 BC but the etiological agent, was first observed at post mortem in 1835 by James Paget a young medical student, on the basis of this observation it was later described and termed *Trichinella spiralis* by Richard Owen. In 1860, Friedreich Zenker found these parasites at autopsy of a female who had a presumptive diagnosis of typhoid fever. He was able to study the pigs that this woman was raising and to establish a link with the ingestion of uncooked pork (17,34, 46,52,).

Epidemiology

Trichinosis is transmitted from one animal to another through ingestion of meat infected with encysted larvae. One hundred fifty species of mammals from different areas of the world may acquire the infection, so that parasitosis may be found at all latitudes. It is a parasitic disease closely related with cultural and dietary habits.

Although its incidence has fallen over the last half century, it still remains a worldwide public health problem, and cases may be unreported or misdiagnosed.

Though endemic throughout the world, *T. spiralis* infection has not yet been reported in Australia and in some islands of the Pacific Ocean (37).

In United States, the incidence of trichinosis has decreased since national reporting began in 1947, but in 1990 there were two outbreaks, highlighting the importance of control measures. In the late 1940's there was an average of 400 cases and 10-15 deaths per year; from 1982 through 1986 incidence declined to an average of 57 cases per year and a total of three deaths. From 1987 through 1990, 206 cases from 22 states were reported to CDC (53,66,68,78). Outbreaks have been reported from Hawaii,(87) Iowa, Wisconsin, Rhode Island and Virginia, stressing the need for ongoing education about the risks of eating undercooked pork (57). Twenty-six cases of travel-associated trichinosis were identified during the 1975-1989 period. Sixty-five per cent of affected patients had travelled to Mexico and Asian countries (14,54).

Outbreaks are still associated with immigrants or ethnic groups that prefer raw or undercooked pork or its products, while wild animals may also act as carriers.

Sporadic cases are not infrequent in northern Canada among the Indians, whose sources are wild land or marine animals while outbreaks have been reported in 1974 (49 cases), 1975 (3 cases) 1976 (31 cases) (1,7)

In Europe the incidence is low due to mandatory inspection of pork for Trichinella. Over the last two decades, outbreaks were related to the ingestion of horsemeat and wild animals infected by *T spiralis* and other species. Among others, outbreaks occurred in France in 1976 (125 cases) and 1985 (400 and 980 cases); in Italy, in 1976 (96 cases), in 1984 (13 cases), in 1986 (300 cases), and in 1988 (48 cases) (30,60). Cases have been reported from Spain, from 1986 to 1990, 430 cases in 13 outbreaks, mostly due to ingestion of boar meat and other wild animals; from Poland from 1976 to 1986, 2953 cases in 135 outbreaks, and from Germany, Austria, former Czechoslovakia and Yugoslavia. In Asian countries such as Thailand, since 1962 the disease has flared up almost every year involving 5400 patients (45). China with 58 cases in 1981 and 86 in 1983 (1), and Japan with 3 mass outbreaks in 1974, 1980 and 1982, whose main source was raw black bear meat (77).

In Lebanon a severe outbreak involving 6440 individuals occurred in 1982 (58); Egypt suffered two outbreaks in 1975 and 1985, mostly due to infected pork, whilst in the rest of Africa the main sources are wild animals (57).

With regard to Latin America, trichinosis has been described both in man and animals. Mexico had 17 outbreaks from 1978-1983 and a total of 108 cases in the province of Zacatecas (1).

In Argentina there are endemic areas throughout the country. On average five outbreaks of trichinellosis take place annually in the province of Buenos Aires (15) caused by consumption of pork and its derivatives that evade sanitary inspection. Reported outbreaks include 129 cases in 1981, 151 in 1982 and 87 in 1983 (1). In the 1986-1993 period, 43 outbreaks with a total of 699 cases were officially recorded (9,12,). During 1994, 386 cases were diagnosed, 711 in 1995 (13) and over 460, with one fatal case, in 1996. Wild animals, such as puma and boar, may also cause disease, but no epidemiological or clinical data are yet available.

Chile reported 167 cases in 1975 and 66 in 1981, with a later drop in the incidence of new infections (26), while in Venezuela and Uruguay incidence seems to be very low (57). Outbreaks were described in 1982 for the first time in Honduras and Costa Rica whereas the remaining Latin American countries have not reported cases.

Control

Efforts to prevent Trichinella infection of man and animals must be directed towards: 1) preventing the consumer from eating infected meat (meat inspection); 2) technological aspects of the manufacture and preservation of meat products (destruction of larvae: heating, freezing, curing and drying); 3) hygienic measures including feeding regulations for pigs; 4) rats and mice control on farms; 5) serological control of animals; and 6) public health education (12,64,70,72)

Several recommendations have been established by the U.S. Department of Agriculture (USDA), World Health Organization (WHO), Office International des Epizooties (OIE) in order to avoid transmission to humans including guidelines for safe freezing and cooking. USDA and Food and Drug Administration have also approved the irradiation of pork which renders muscle larvae nonviable (57).

Etiological agent and life cycle

Trichinella spiralis belongs to the phylum Nematoda. They are viviparous worms with a cylindrical, pseudocelomic cavity, complete digestive tube and separate sexes. The class is Aphasmidea, order Enoplida, family *Trichinelloidae*, genus *Trichinella*. Several species are now recognized by DNA probes and alloenzyme markers, also differing with regard to distribution, infectivity, pathogenicity and reservoir (61).

Species include *T. spiralis* (T1) and *T. pseudospiralis* (T4), which are both cosmopolitan; *T nativa* (T2) in the Arctic pole; *T. nelsoni* (T7) in equatorial Africa and *T. britovi* (T3) in temperate climates. The major host reservoirs for *T. pseudospiralis* are mammals and birds and this is the only species that does not encyst (57).

Adult male worms are smaller than females, the former measuring 1.10 to 1.6 mm in length, and the latter 1.5 to 3.3 mm. After human ingestion of uncooked infected pork, pepsine and hydrochloric acid digest the cyst capsule and larvae are released to lodge in the upper small intestine and penetrate the epithelial lining, where they continue their develop-

ment; after four moults they become sexually mature adults in 2-3 days. After mating, viviparous females begin to deposit newborn larvae 4-5 days after ingestion of infected meat which generally continue to be laid for some weeks depending on the host, until the female adult worm is rejected from the intestine (19). In rodents it has been estimated that the number of larvae per female worm range from 1500 to 50,000 according to *Trichinella* species. The intestinal phase varies in relation to host species, ranging from 10 days to several weeks. Newborn larvae issuing from the intestinal wall enter lymphatic vessels and mesenteric venules reaching the posterior vena cava through the thoracic duct or portal vein, invading the heart and lungs, to migrate through the circulation to invade tissues, where despite transient infections they generally die in a short period of time. When they reach striated muscle they become encysted and may remain viable for 5 to 10 years. Newborn larvae prefer active muscle groups such as diaphragm, masticatory, tongue, intercostal and pectoral muscles, but also back and lumbar region muscles (20).

After penetrating muscle fibers, larvae induce changes in the host cell and stimulate capsule formation around 2 weeks pi. Newborn larvae continue their growth and reach 1 mm in length, when they are found rolled up into a spiral form within the completed capsule at 4-5 weeks pi. The anterior half of the larva presents stichosomes, with a row of roughly 50 discoid cells or stichocytes which are secretory and their product highly antigenic eliciting through the capsule host protective immunity and proving useful for immunodiagnosis (50,63)

There are 2 cycles of transmission, one domestic and the other wild. Transmission from pigs to humans of *T. spiralis* is known as the domestic or synanthropic cycle. The domestic cycle also involves other animals such as rats, dogs and cats. *T. spiralis* may also infect other hosts, which are herbivores such as horses infected by rat carcasses incorporated to their feed (5). In Arctic regions the main source of infection is the ingestion of meat from wild animals such as walrus, seal and polar bear, and in Africa from wild canids and felids (1). Others as the fox, boar and small rodents may also take part in this cycle in temperate areas. Although transmission from pigs to wild animals has been demonstrated, the reverse is unproven (57).

All species have a life cycle with complete development in a single host and are found initially in adult form in the host intestine; subsequently they reach muscle where larvae are encysted. In order to initiate a new cycle and complete their development, *T. spiralis* muscle-encysted larvae require to be ingested by a new host which may belong to the same species or to a different one.

Clinical Course and Manifestations

Host factors, such as health status, immunocompetence, sex and age, as well as the amount of ingested larva and the *Trichinella* species influence the clinical course (59). The incubation period varies from a few days to one or almost two months. The clinical course may be asymptomatic or subclinical in a high percentage of human infections (46).

The enteric phase is due to the presence of larvae within the villi of the small bowel where they undergo their four moults and turn into adult worms. The first and second week post ingestion of the infected meat is characterized mainly by diarrhea, though constipation, vomiting and abdominal pains may also occur. Such symptoms are present only in one third of the cases although they are more frequent in infections acquired in the Arctic (49).

The acute phase, also termed *trichinellotic* syndrome and lasting approximately two months, is mainly characterized by eosinophilia, fever, myalgias, and periorbital as well as facial edema and represents an allergic inflammatory response, with subungueal petechiae as a frequent finding. Larvae are unable to encyst in organs other than striated muscle. The course may be mild, moderate or severe. Several organs may be involved by migration and presence of parasites in tissues as well as by immune mediated inflammatory infiltration.

Cardiovascular involvement features myocarditis, heart failure and nonspecific EMG changes. Lung involvement may be preceded by respiratory symptoms such as coughing and dyspnea, due to involvement of the diaphragm followed by pneumonitis and/or infarction resulting from larval passage or presence in pulmonary vessels.

Central nervous system (CNS) involvement has been estimated at about 10 to 24 % of symptomatic cases (16,39,46), while it has been considered as low as 0.75% in more recent reports (3,25). A spectrum of signs and symptoms have been reported, such as headache, vertigo, delirium, insomnia, seizures, aphasia, psychiatric changes, meningeal involvement, eye muscle/orbit involvement and cranial nerve palsies, as well as evidence of focal signs which are mainly observed after the third week post infection. El Koussa et al (24) described a rare case of sinovenous thrombosis and reviewed four other cases (27,31,7,39). On occasion, peripheral nerve involvement has been observed (16,46,52).

CT brain scanning discloses multiple small hypodense lesions in hemispheric white matter with ring-like enhancement following intravenous contrast (47), as well as small cortical infarcts (29) while bilateral high-signal intensity lesions of the white matter have been demonstrated by MRI (25).

The mortality rate is generally low and death is mainly due to heart failure or CNS involvement in 5 to 10 % of infected individuals (46). Disseminated intravascular coagulation may be a postmortem find-

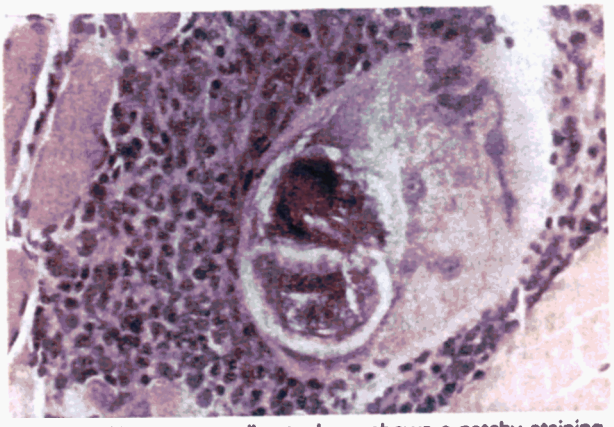

Figure 1. Host nurse cell cytoplasm shows a patchy staining, cell capsule as well as larval external cuticle remain unstained. Hematoxylin and eosin x 400.

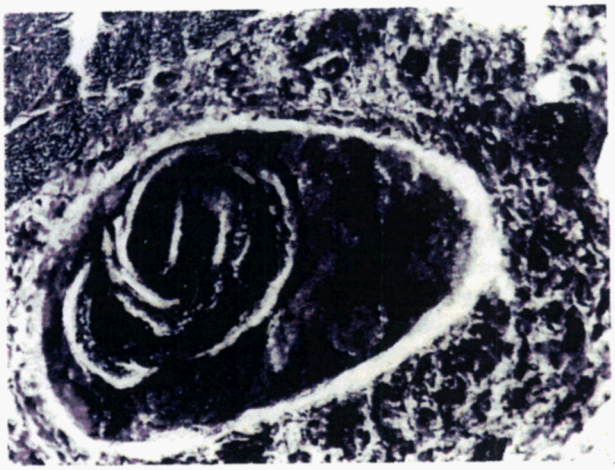

Figure 2. Intense activity of both larva and host nurse cell cytoplasm, the latter with patchy staining, capsule remains unstained. NADH-TR x 400.

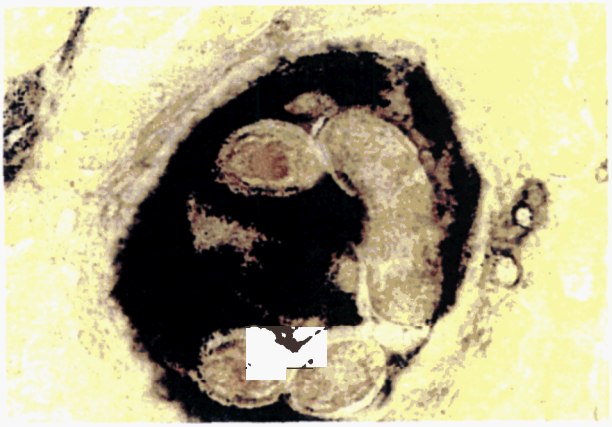

Figure 3. Strong alkaline phosphatase activity in host nurse cell cytoplasm, larva and capsule remain unstained. Perymisial staining is evident on the upper left of the figure. Alkaline phosphatase x 400.

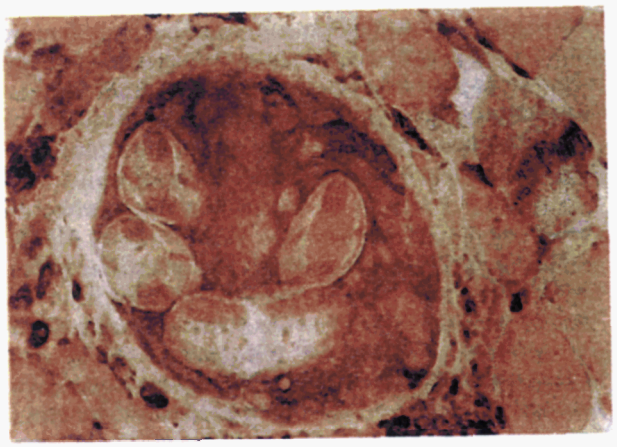

Figure 4. Intense non-specific esterase activity in host nurse cell cytoplasm, there is also some staining of the larva. Non-specific esterase x 400.

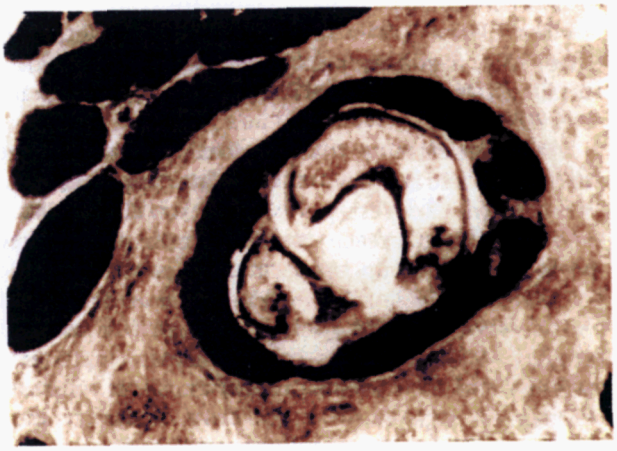

Figure 5. Strong ATPase pH4.6 activity in host nurse cell cytoplasm; the larval internal muscle layer is also strongly reactive while parasite cuticle and external capsule remain unstained. ATPase pH.4.6 x400.

ing. Improved therapy has contributed to decrease the mortality rate, which was 0.31% during the outbreaks due to horsemeat trichinellosis in Western Europe in the last decade (Italy 1975/1986, France 1976/1985) (11,51,57). Convalescence lasts from months to years and most cases recover completely, with larval calcification. Trichinellosis has also been reported in patients receiving immunosuppressive therapy (22,40).

Muscle involvement is characterized by myalgias and proximal weakness, which are more intense a few weeks post infection; erythematous changes are usually associated mimicking dermatomyositis. Contractures may develop and even affect jaw opening (36). EMG shows both myopathic changes and fibrillation potential as in idiopathic polymyositis.

Diagnosis is not difficult when Tichinellosis occurs as an epidemic disease, but it may be very difficult in sporadic cases. Typhoid fever, meningitis, polymyositis, dermatomyositis and periarteritis nedosa are the main differential diagnoses.

Clinical Laboratory Examination

Blood assay discloses severe leukocytosis in the acute phase, particularly eosinophilia characteristic of helminthiases, as well as up to 10-fold increases in total Ig E serum levels. Muscle enzymes such as creatinkinase and lactic dehydrogenase values are also raised but tend to decrease at 6 weeks pi.

Cerebrospinal fluid (CSF) examination may be useful in cases with CNS symptoms, larva being recovered in 8% to 28 % of cases (8).

Charcot-Leyden crystals from eosinophils may be found in stools but worms are exceptional.

Both humoral and cellular immune responses to *T. spiralis* antigens are evident during infection and several serological assays have been developed to ensure sensitivity and specificity, some of which may be readily applied to field studies.(12,48,57,64). Prompt treatment should be carried out on clinical suspicion and laboratory findings, since seroconversion only becomes detectable after the third week pi.

Specific serum antibodies are detected by complement fixation, bentonite flocculation, latex agglutination, indirect hemagglutination, counterimmunoelectrophoresis, indirect immunofluorescence and enzyme-linked immunoabsorbent assay (ELISA), as well as the competitive inhibition assay (CIA). Circulating antibodies may be detected by newly developing immunoradiometric (IRMA) or enzymatic capture techniques (EIA), which employ specific monoclonal antibodies for *T. spiralis* L1 (muscle infective) larva. Circulating immunocomplexes are determined by complement fixation binding or precipitation with polyethylenglycol (50).Cell immunity may be evaluated by Bachman's intradermal reaction using muscle larva as antigen, but it proves poorly specific.

Molecular biology procedures are under active research worldwide and a consensus on *T. spiralis* antigens and antibodies was reached in 1990 providing *Trichinella* antigen nomenclature and monoclonal antibody characterization. Interestingly, *T. spiralis* larvae group I antigens (TSL-1) arising from the stichosome seems to be immunodominant and may play a role in host nurse cell-larva interrelationship, though its biological function remains obscure (4,33). DNA probes and PCR technology have been developed to specifically identify domestic *Trichinella* from wild isolates (76); in the near future these techniques may be also applied to meat screening.

Parasitological investigation by muscle biopsy affords the most reliable diagnosis but only as from the third week pi. If necessary (field studies) specimens may even be studied by compression between slides and light microscopy observation, xenodiagnosis consisting in feeding laboratory animals with biopsied muscle and searching for carcasse larvae 4-5 weeks later may be also performed.

Pathology and Pathogenesis

Trichinella infection induces traumatic and immunological alterations, the former due to larvae and adult worms and the latter to parasite antigens.

At intestinal level there is severe mucosal and submucosal inflammation, while in the blood stream parasite antigens are released by migrant larvae. Contact with immune system cells (granulocytes, mast cells and platelets among others) leads to immediate hypersensitivity, clinically reflected in facial edema, fever and conjunctival hemorrhage resulting from small vessel vasculitis. Larvae invade striated muscle where they encyst.

On reaching the heart parasites induce often transient myocarditis releasing their excretion-secretion products, but in the CNS the damage may be more severe when larvae traverse the meninges. Active larval metabolism induces both local and generalized immediate hypersensitivity reaction due to a wide spectrum of antigens according to larval stage, number and host species.

Central Nervous System. Eosinophilic meningoencephalitis, glial hyperplasia, small hemorrhagic foci in the white matter and noninflammatory vascular necrosis, as well as arteriolar and small capillary thromboses together with small ischemic lesions even in the absence of larvae have been described. On occasion larvae have been found in granulomatous areas in brain, meninges and CSF or as emboli within small vessels. Brain lesions may involve both gray and white matter, cerebellum, pons and spinal cord. (16,29,35,46,56,67).

CNS involvement has been variously attributed to diffuse tissue damage resulting from larval migration during the two weeks following infection (24), to vascular obstruction secondary to the presence of larvae and inflammatory infiltration (35,46), to toxemia due to larvae or their products (16), or to eosinophilic infiltration and its effects (25,29,55, 71,72). However, larvae cannot be detected in many pathological lesions, indicating immune mediated pathogenesis or reaction to toxins (52) and resulting vascular damage. Furthermore, trichinosis may induce endothelial cell damage mediated by tumor necrosis factor (TNF)α which in turn leads to eosinophil toxicity not only towards human endothelial (29) but also against neural tissue (52).

Chemotactic factors such as eosinophil stimulation promoter, ECF-A and cytokines such as IL-5 produced by the TH2 subset of CD4 T cells have been held responsible for eosinophilia culminating in larval death and concomitant vascular injury (28,73).

Although raised total IgE is characteristic of helminthic infections, experimental evidence is inconclusive as regards a protective role against *T. spiralis* infection. (57,74).

Muscle Biopsy. Muscle biopsy is the most reliable diagnostic procedure and was thoroughly used before serological tests became available.

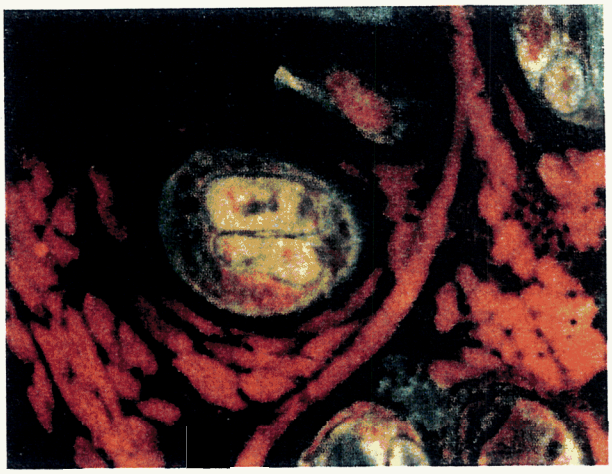

Figure 6. Fluorescent encysted larvae are clearly discernible from the surrounding muscle fibers which are counterstained red. Cryostat sections incubated with positive human serum for *T. Spiralis*. Iindirect immunofluorescence, antihuman IgG labeled with fluorescein isothiocyanate counterstained with Evans Blue x 400.

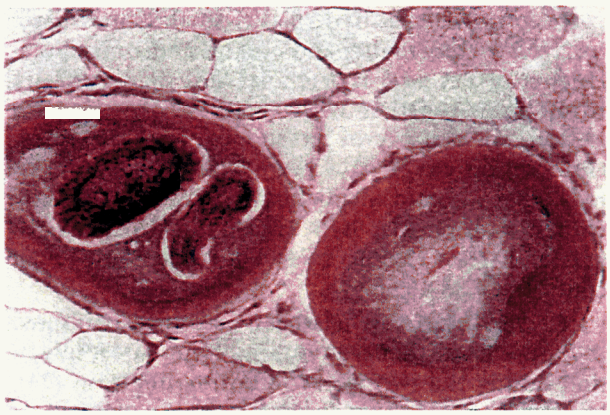

Figure 7. The capsule of the encysted larva is strongly PAS positive; the larva external layers are also stained. Periodic Acid Schiff-Hematoxylin x 400.

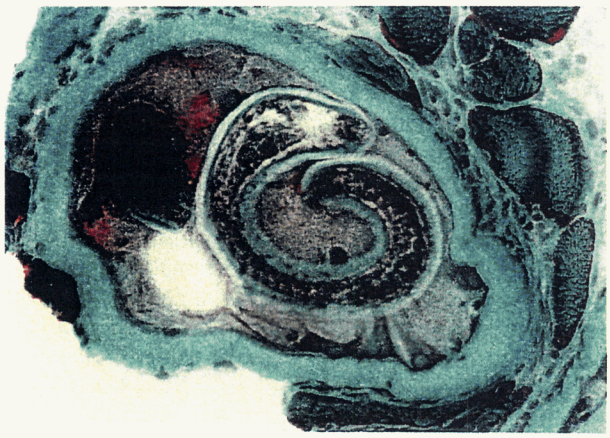

Figure 8. Increased subsarcolemal and intermyofibrilar staining in type 1 muscle fibers, host nurse cell cytoplasm stains patchy reddish and greenish while cell capsule stains green. Larva is coiled within the host nurse cell cytoplasm. Gomori's trichrome x 400.

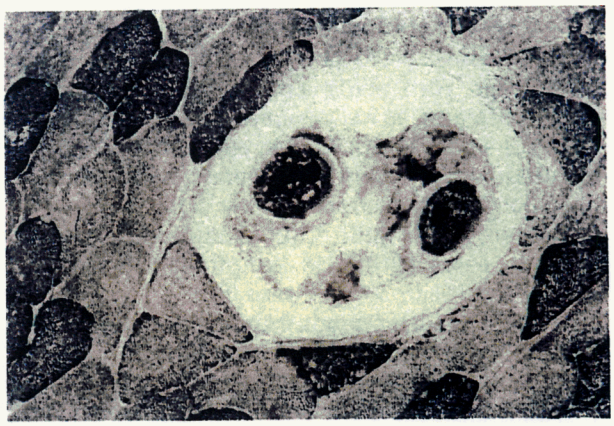

Figure 9. Larvae exhibits high oxidative activity with SDH while host nurse cell cytoplasm has a patchy staining, capsule as well as larval cuticle remain unstained x 100.

Biopsy should be performed in a clinically affected muscle presenting pain and/or swelling. The number of positive fibers is related to infection level while a negative result does not exclude the disease due to the multifocal pattern of larval distribution.

With regard to findings in humans, our own observations are mostly gleaned from an Argentine outbreak in 1983 in Esquel, Argentina. We reviewed paraffin embedded muscle biopsies (biceps or deltoid), performed in seventeen out of forty patients in a local hospital, while a further two were frozen in isopentane in liquid nitrogen and cryostat sections stained or reacted by routine histological and histochemical techniques, as well as immunostained with monoclonal antibodies CD20, CD45, CD45RO (Dako).

We also studied tongue muscle from 10-week-old female Swiss mice infected orally with 300 muscle larvae (ML) of *T. spiralis* at 30 days pi together with a mouse tongue control muscle. Cryostat sections of frozen muscle were incubated with positive human serum for *T. spiralis* and tested by indirect immunofluorescence. Frozen sections were also studied by histological and histochemical techniques and immunostained with a rabbit polyclonal antiserum for dystrophin (kindly provided by Dr. A. Engel).

Human muscle biopsy: At light microscopy there was relatively good preservation of structure with multiple small perivascular as well as endomysial inflammatory foci, consisting mainly in monocytes, T lymphocytes, a few plasmocytes and eosinophils. In some foci neutrophils were also observed. Occasionally, mononuclear cells invaded muscle fibers. Serial sections disclosed one or more encysted larvae within a modified muscle fiber with heterogeneous, patchy cytoplasmatic staining, in areas basophilic, surrounded by an eosinophilic, PAS-positive capsule. So-called "nurse cells" containing encysted larvae were multinucleated with prominent nucleoli, more evident in paraffin longitudinal sections tangential to the larvae, where more host cell

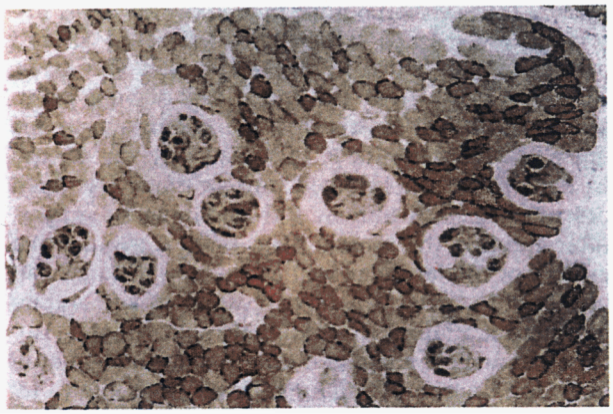

Figure 10. Cytochrome-c oxidase activity exhibits a pattern similar to SDH. Cytochrome -c oxidase a x 100.

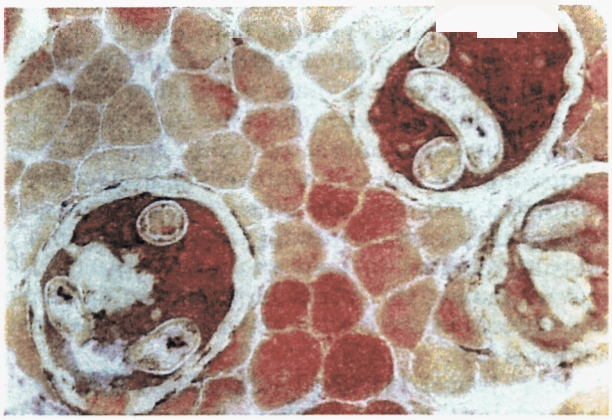

Figure 11. Strong non-specific esterase activity of host nurse cell cytoplasm, larvae shows granular staining, both cuticle and capsule remain unstained. Non specific esterase x 400.

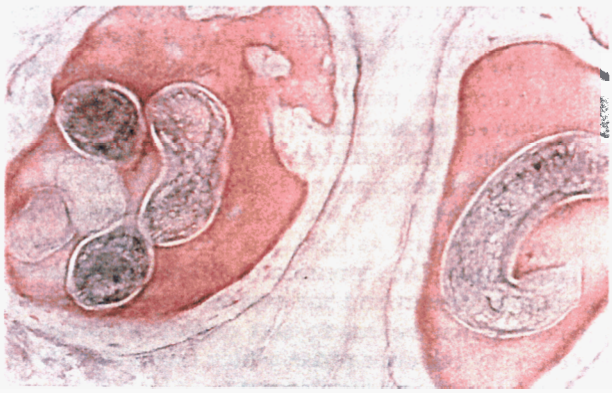

Figure 12. Intense acid phosphatase activity in host nurse cell cytoplasm, larvae as well as capsule remain unstained. Acid phosphatase-Hematoxylin counterstain x 400.

cytoplasm could be discerned. Inflammation surrounding encysted larvae ranged from minimal to prominent nodular cellular infiltration (Fig. 1). The body of the larva appeared coiled within the host nurse muscle fibers, or as two or three larval profiles, a peculiar appearance probably due to a U-turn of a single one. A central cavity corresponding to the rudimentary digestive tube together with a thin muscular coat beneath a superficial cuticle could be discerned. This structure was more evident in experimentally infected mice, or in human cases when serial sections were performed or there were several encysted larvae. Occasionally, irregular amorphous, hyalinized material suggestive of a wrinkled fibrotic capsule was observed corresponding to degenerated or dead larvae undergoing reabsorption.

The possibility of a remote myopathy has been advanced, due to the fact that inflammatory, degenerative and regenerative changes may be observed at some distance from encysted larvae, so that the role of a toxin or hypersensitivity mechanism has been entertained (2). In experimentally induced trichinosis in guinea pigs and serial section technique, Drachman and Tunebay (23) were able to demonstrate trichinae undetected by routine techniques in a number of myofibers. Gherardi et al (32) in muscle biopsy studies from patients afflicted by a trichinosis outbreak in France found that inflammatory cells were mostly monocytes and T lymphocytes mainly of the suppressor/cytotoxic phenotype.

Gross and Ochoa (36) reported vigorous larval staining with NADH-TR and myosin ATPase activity at pH 4.6 in the acute stage of infection, indicating that larvae were alive. An increase in host nurse cells acid as well as alkaline phosphatase activity has been described, at ultrastructural level the latter has been found within transverse tubules, concentric membranous structures and regions of the plasmalemma (10). In cases examined by the authors of this chapter, host nurse cell histochemistry showed patchy heterogeneous sarcoplasmic appearances in NADH-TR (Fig. 2), SDH and Cytochrome-c-Oxidase, whilst both alkaline (Fig. 3) and acid phosphatase, non-specific esterase (Fig. 4) as well as myosin ATPase at pH 9.4, 4.6 (Fig. 5) and 4.2 showed more homogeneous intense reaction. In the perimysium, alkaline phosphatase showed intense reaction as described in inflammatory myopathies.

Experimental infection in mice: Infected mouse tongue muscles showed 6 to 15 encysted larvae per transverse cryostat section, with well preserved muscle structure. Larvae strongly react with positive human serum by indirect immunofluorescence (Fig. 6). Many encysted larvae at this stage lacked peripheral inflammatory reaction, which was slight or moderate only around a few of them. Small foci of inflammation were observed at some distance, probably related to other encysted larvae not appearing in the section. The capsule surrounding the host nurse cell is strikingly PAS-positive (Fig. 7). An increase in oxidative activity in type 1 fibers with a ragged red appearance in Gomori's trichrome (Fig. 8) was observed, unlike in human limb muscles, but this was identical to tongue muscle of control mice. In histochemical studies of tongue muscles from infected mice, host nurse cells appeared green with red

areas when stained with Gomori trichrome (Fig. 8), whilst NADH-TR (see Fig. 2), SDH (Fig. 9) and cytochrome-c-oxidase (Fig. 10) showed patchy areas of intense activity. Non-specific esterase (Fig. 11) and both alkaline and acid (Fig. 12) phosphatase activity were also positive. Myosin ATPase reaction was strongly positive at pH 9.4, 4.6 (see Fig. 5) and 4.2.

Larval reaction was similar in both human and mouse muscle, appearing green with Gomori trichrome, strongly reactive with with NADH-TR and irregularly positive with SDH and Citochrome-c-Oxidase. Both acid and alkaline phosphatase reactions proved negative, whilst a slightly positive granular pattern was observed with non-specific esterase. Larvae were also readily disclosed by ATPase at 9.4, 4.6 and 4.2, their cuticle remaining unstained whereas the internal muscle layer was strongly reactive. Mouse muscle dystrophin immunostaining was preserved in uninfected muscle fibers, as it was in controls, but no sarcolemmal staining in the host nurse cells-capsule interface could be seen, despite discernible background within the host nurse cell sarcoplasm. This effect was probably due to incipient degeneration. Dystrophin antiserum seemed to react with the muscle layer of the larva.

Host Nurse Cell and Larva Interrelationship

Host nurse cell histological appearance and histochemical profile differ markedly from striated muscle cells. Slightly basophilic sarcoplasm as well as increased enlarged nuclei with prominent nucleoli resemble regenerating muscle fibers. Increased activity of both acid and alkaline phosphatase are also indicative of degeneration and regeneration (6).

The described histochemical profile may be due to replacement of myofilaments by an increase in sarcoplasmic reticulum (SR), mitochondria and/or T tubules as well as enhanced lysosomal activity. The capsule, a host derived product, rich in mucopolysaccharides, appears as a thick barrier protecting the host nurse cell-larva complex from immune attack. Dystrophin was not disclosed at the capsule/host nurse cell interface. Studies on experimental infected mice are under way in order to ascertain whether other proteins take part in capsule formation, such as different types of collagen.

Adaptive changes have been documented by Despommier (18) in infected muscle fiber host nurse cells at ultrastructural level following experimental infection in mice with injections of newborn larvae. At days 3-4 pi, a subsarcolemmal space develops containing increased glycogen, mitochondria and vesicles, with myofilament disarray and enlarged nuclei migrating to an internal position. At day 5, sarcomeres are disorganized with an increase in SR and transverse tubules, with a progressive replacement from day 8 through 10 of myofilaments. Later, the plasma membrane becomes hyperinvoluted and there is an increase in basal membrane thickness, while a host-derived double membrane develops surrounding the larva, adjacent to increased SR and mitochondria. Thus, in order to reach the larva, nutrients must cross the nurse cell plasma membrane, nurse cell matrix and the membrane at the nurse cell-larva interface. Increased vascular density in the vicinity of infected muscle fibers has been described in both human and experimental studies. After entering the muscle fiber, there is exponential growth of the larvae as well as changes in nurse cell nuclei. As from day 5pi, larva resumes growth and by day 20 pi it attains a 10-fold increase in volume (18). Host cell nuclear changes during synchronous mouse infection with *T. spiralis* were described by Despommier et al (21), showing that nuclear enlargement is gradual from day 0 to 7, increasing in volume from day 7 to 8. An average of 40 enlarged nuclei per nurse cell was established, host nuclei involved being those of mature muscle cells.

Changes that occur in skeletal muscle fibers induced by the larva in order to become a nurse cell are unique for *Trichinella spiralis* infection. Molecular signals from each muscle cell and harbored larva controlling this host cell redifferentiation have been postulated (62), and are under study. Nurse cell remains alive together with the larva for a long time, probably decades in men and a life-span in the infected mouse (18). A state of permanent regeneration induced by the larva in the nurse cells has been advanced (65).

D. P. Jasmer (41,42,43,44), updates infection-induced host muscle changes. On one hand, it acquires non-differentiated muscle features such as a circulatory network surrounding the infected cell and development of a collagen capsule, as well as increases in lysosomal enzyme activity, in rough endoplasmic reticulum and enlarged internal nuclei with prominent nucleoli. On the other hand,. there is also a loss of differentiation because myofibrillar proteins have proved undetectable in chronically infected muscle cells. These changes seem to be regulated at muscle gene transcription level. There is also a cell cycle repositioning, as the muscle cell re-enters the cell cycle, replicate its DNA and becomes suspended in G2/M stage. The repression of differentiated skeletal muscle features and the expression of a different phenotype may be due to host regulatory pathways governed by cell cycle phase, which might contribute to larval growth and development.

Treatment

Treatment of human trichinellosis is based on specific chemotherapy directed against the adult parasite and migrating larvae together with symptomatic treatment. Therapeutic action on encysted larvae is controversial. Levamisole and Pyrantel may be used only against adult *Trichinella*. Thiabendazole, albendazole and mebendazole are usually administrated (12,64).

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