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With 3 figures in the text

The pathogenicity and development within the host fish of *Myxobolus cyprini* Doflein, 1898

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SUMMARY

Myxobolus cyprini has, until now, been considered an 'organo-cosmopolitan' parasite of the common carp (*Cyprinus carpio*) and less frequently of other carps, producing spores in various organs in small plasmodia and possibly in cysts. The present observations of naturally infected common carp fry and two-summer carp have revealed that *M. cyprini* is a specific muscle parasite, developing intracellularly in the muscle fibres of the skeletal muscle. The sarcoplasm of the infected muscle fibres is filled with developmental stages, followed by spores of *M. cyprini*, which are held together in a 1–1·5 mm long pseudocyst by the sarcolemma of the muscle fibre. After maturation of the spores and disintegration of the pseudocysts the spores are transported in the bloodstream to different parts of the body where they are retained in the capillaries.

INTRODUCTION

Myxobolus cyprini is one of the longest-known parasitic protozoa of the common carp (*Cyprinus carpio*). Since its first description, numerous authors have demonstrated its presence in common carp stocks, and most of these authors have attributed pathological significance to it. In spite of its frequent prevalence, no detailed studies have been performed on organ specificity and pathology, and the literature contains contradictory data in this respect.

Most authors have considered *M. cyprini* a species able to develop in different organs. Plehn (1924), Shulman (1966) and Bauer, Musselius & Strelkov (1981) described spore formation in different tissues in diffusely scattered plasmodia, while Kocylowski & Myaczynski (1963) and Schäperclaus (1954) found larger cysts in various locations, including the musculature of the fish.

Numerous hypotheses have been put forward concerning the pathogenicity of *M. cyprini*. The original notion that *M. cyprini* might have a role in the aetiology of carp-pox was disproved by Plehn (1924). The supposition of Spiczakow (1935) that mass invasion of carp by *M. cyprini* results in a so-called malignant anaemia was accepted by Shulman (1966), Kocylowski & Myaczynski (1963) and Bauer *et al.* (1981), despite the fact that, according to Schäperclaus (1954), the symptoms observed in severe *Myxobolus* infection can probably be attributed to a simultaneously occurring infectious dropsy.

The present paper reports our results showing that *M. cyprini* is actually a muscle parasite and that the diffusely occurring spores which are present in other organs are a result of a secondary process.

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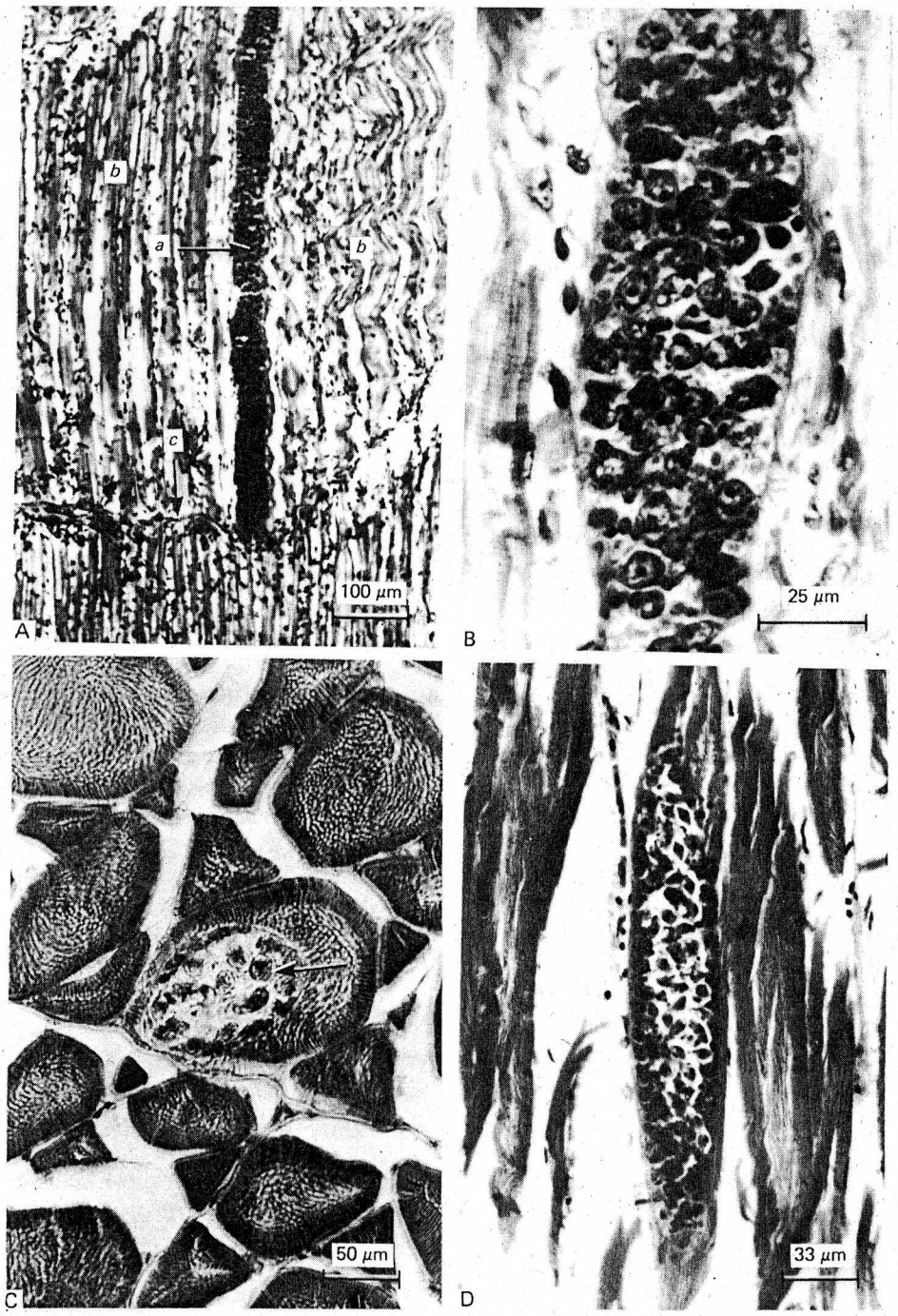

Fig. 1 A. *Myxobolus cyprini* pseudocyst in the skeletal muscle of the common carp. *a*, Pseudocyst, *b*, muscle fibres; *c*, myosepta. (Haematoxylin and Eosin.) B. Young developmental stages of *M. cyprini* within the muscle fibre. (H. and E.) C. Cross-section of muscle fibre infected by *M. cyprini* spores (arrow). (Farkas-Mallory's staining.) D. Semi-mature pseudocyst with spores in its centre, with developmental stages near the sarcolemma. (H. and E.)

MATERIALS AND METHODS

Common carp fry and two-summer common carp stock were obtained from one of Hungary's large fish farms. From March 1982 ten fish were dissected and examined for parasites at 2-week intervals. In total, 370 common carp fry and 220 two-summer common carp were examined. In addition to these, fish from other stocks, severely infected by *M. cyprini*, were examined occasionally. The fish were killed in the laboratory. Kidney, liver, spleen and muscle were examined fresh, in impression smears and drops of blood under coverslips, and intestinal and gill scrapings were also examined.

The organs of 2 fish from each experimental group were fixed in buffered formalin, cut by freezing-microtome and stained with haematoxylin and eosin. After fixation of tissues from severely infected fish in Bouin's solution and embedding in paraffin, sections were made and stained with haematoxylin and eosin and by van Gieson and Farkas-Mallory staining.

As well as these specific observations, the records from previous routine diagnostic examinations of fish were used to compile data on the organs affected.

RESULTS

M. cyprini spores were seen scattered in haemopoietic islets of the kidney, spleen and liver and also in the gills, intestinal wall, skin and musculature of common carp aged more than 3 months. The prevalence in common carp fry reached 57 %, and in older fish it was between 85 and 95 %, the intensity varying according to the ponds. The first developmental stages of *M. cyprini* were seen in the skeletal muscle of 3-month-old fry in August, at which time the entire sarcoplasm of infected muscle fibres was filled with developmental stages. The parasitic mass was held together by the sarcolemma and appeared as an elongated, cylindrical pseudocyst which measured 1–1·5 mm in length and 0·05–0·1 mm in width (Fig. 1 A, B). The length of the pseudocyst was the same as that of the individual muscle fibres and extended between two adjacent myosepta. In larger fish there were larger pseudocysts. In cross-sections it was easy to observe that the parasites developed intracellularly in the central part of the sarcoplasm, without true cyst formation (Fig. 1 C). Young spores first appeared at the end of August. They occupied the central part of the parasite cylinder where they were surrounded by early developmental stages squeezed against the remaining narrow band of sarcoplasm or directly against the sarcolemma (Fig. 1 D). In September, some muscle fibres were completely filled with spores, some of which were released from disintegrated muscle fibres and formed islets in the intermuscular space (Fig. 2 A). At this time, the first spores had appeared singly or in groups of 2–10 in the capillaries of the gills, skin, intestine and of other inner organs. In shape and size, the spores were as described by Shulman (1966).

In the majority of overwintered fry and in older fish, early developmental stages (pseudocysts) in the muscles and spores scattered in various organs were found simultaneously.

No local host reaction was observed around the spores while they were retained within the muscle fibres. When spores were released from the disintegrated muscle fibres, most of them disappeared rapidly, and the remainder were surrounded by epitheloid-type cells (Fig. 2 B), and then by connective tissue cells (Fig. 2 C). The 'host reaction' which developed around spores which had been carried to various organs differed between

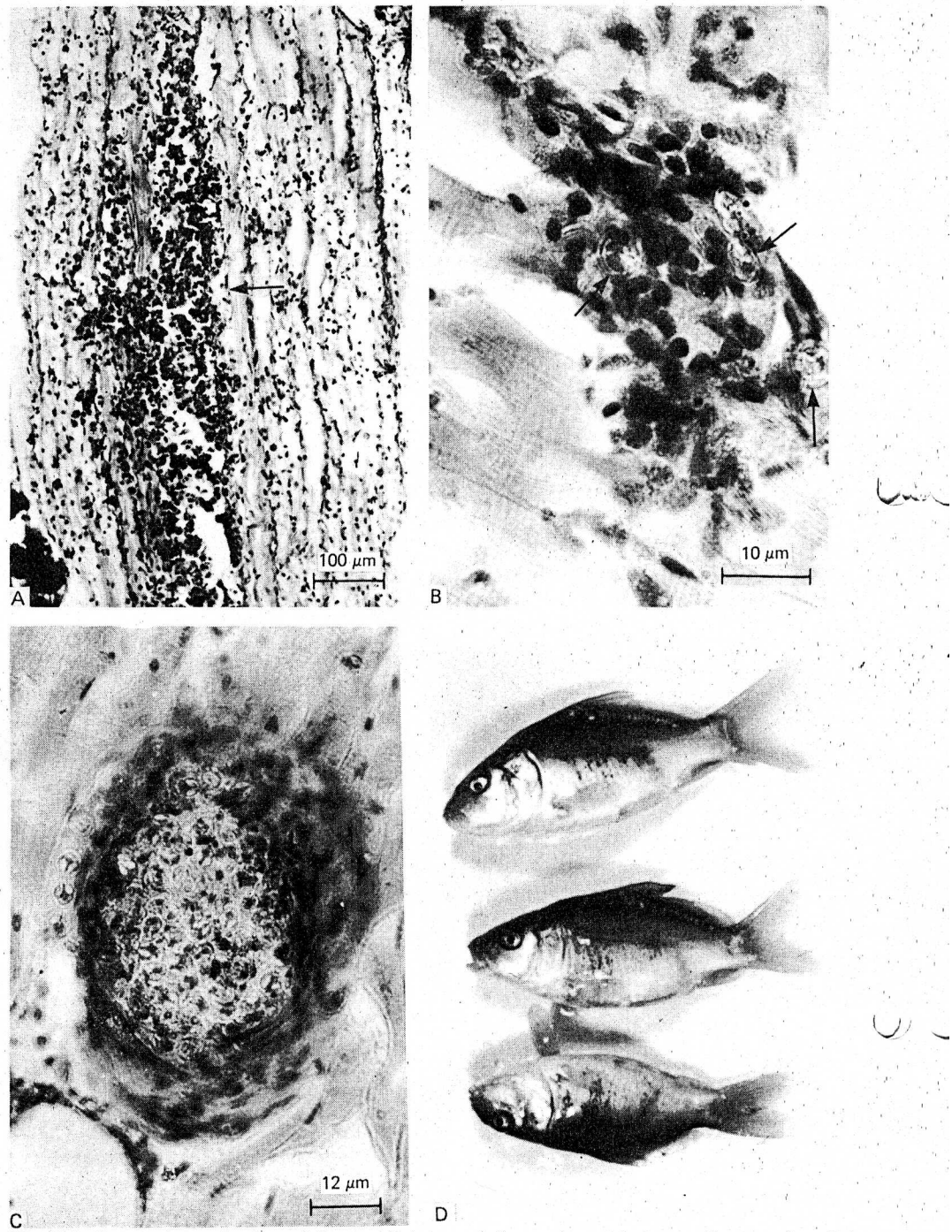

Fig. 2A. Spores (arrow) released from a disintegrated pseudocyst between the muscle cells. (H. and E.) B. *Myxobolus cyprini* spores (arrow) stuck among muscle cells and surrounded by epitheloid cells. (H. and E.) C. *M. cyprini* spores surrounded by connective tissue cells in the muscles of the common carp. (H. and E.) D. Haemorrhages in the skin of common carp fry, caused by spores stuck in the capillaries (slightly smaller than natural size).

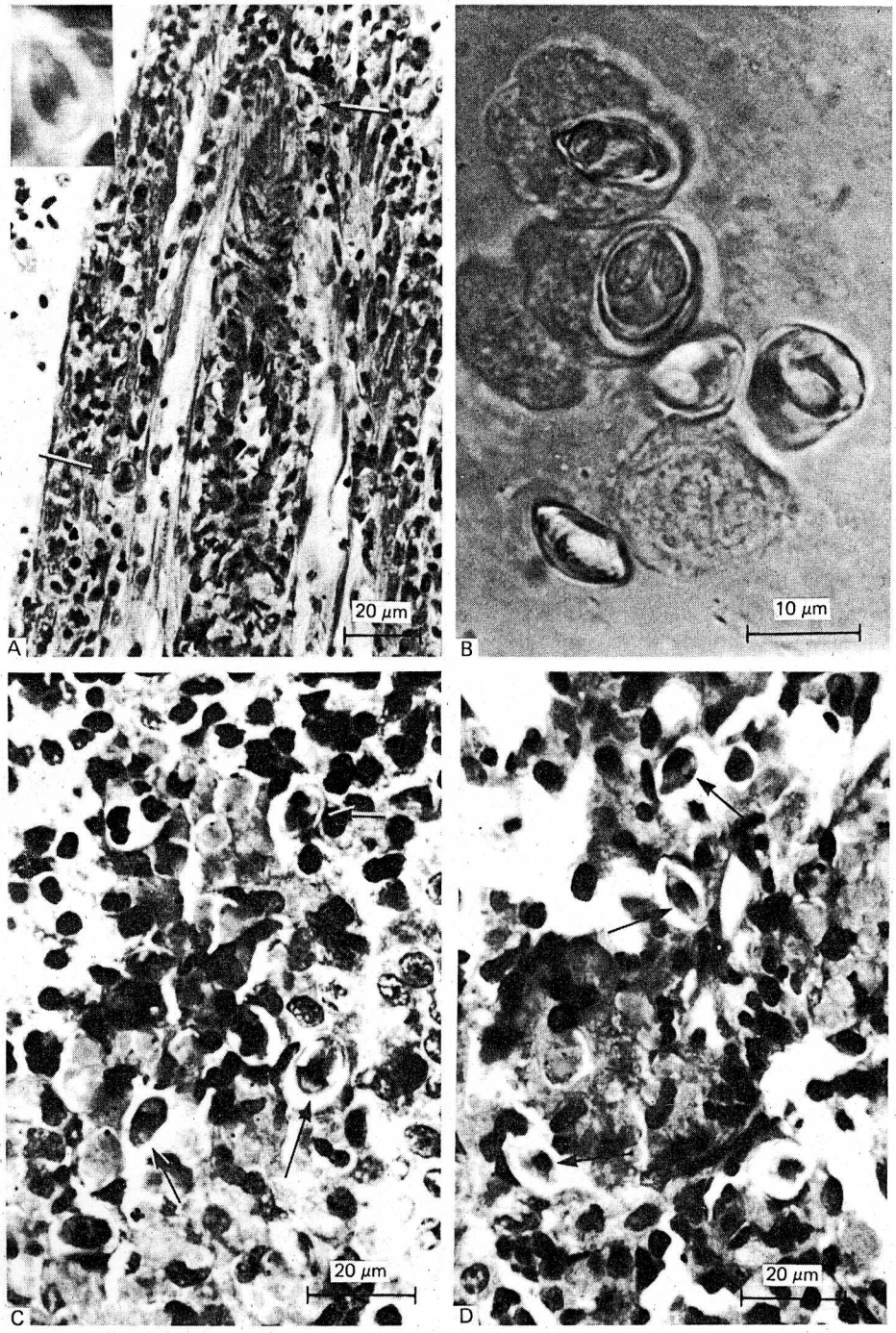

Fig. 3A. Spores (arrow) stuck in the gill capillaries. Insert, a magnified spore. (H. and E.) B. Spores released in yellow bodies in the lumen of the intestine. C. Spores (arrow) accumulated in the macrophage centres of the liver. (H. and E.) D. Deformed spores (arrow) in the macrophage centres of the kidney. (H. and E.)

organs: spores retained in the capillaries of the skin and gills (Fig. 3A) resulted in congestion, occlusion of capillaries and local necrosis (Fig. 2D). Necrosis started to develop also around spores retained in capillaries of the intestine, and the spores were released into the intestinal lumen surrounded by host cells, in the so-called 'yellow bodies' (Fig. 3B). It was characteristic of spores located in the intestinal, gill and skin capillaries that they were released into the environment within necrotic cellular elements. Spores located in the liver, spleen and kidney capillaries were transported to the melano-macrophage centres of the haemopoietic tissues (Fig. 3C) where they were surrounded and lysed by macrophages. In advanced stages of infection the deformation of spores was easy to observe (Fig. 3D).

Some data were obtained on the pathological role of *M. cyprini*. Intensive infections in muscles were observed in numerous cases but there were no signs of external morphological changes or muscle dysfunction. However, infected muscle fibres consistently underwent necrosis and the granulation tissue, surrounding the remaining spores, disrupted the anatomical and functional unity of muscle fibres.

The spores released from disintegrated muscle fibres and which appeared in the various organs seem to play a more important pathogenic role. In addition to the local necrosis caused by the spores, considerable enlargement of the macrophage islets of the spleen and kidney was a common finding and, in one instance, a stock of fry showed external signs. From the beginning of October to the end of November, about 10% of these showed swelling of the abdomen, exophthalmus, reddening of the skin (Fig. 2D), abdominal dropsy and swelling and hydropic degeneration of the viscera. Bacteriological and virological examinations gave negative results but strikingly large numbers of diffusely distributed *M. cyprini* spores were demonstrated in the kidney, spleen and liver. Spores were found in large numbers also in the gills, skin, intestine, and even in the blood. The muscles contained spore masses released from disintegrated pseudocysts. On histological examination, in addition to those in the melano-macrophage islets of the kidney, spleen and liver, spores were observed in capillaries of the peritoneum, gills, pancreas, skin and intestine. The clinical and histological picture resembled in all respects the malignant anaemia of common carp, described by Spiczakow (1935), Kocylowski & Myaczynski (1963) and Bauer *et al.* (1981).

Besides *M. cyprini*, spores of two additional *Myxobolus* species were found in the common carp during the present work.

Cysts of *M. basilamellaris* Lom et Molnár 1983 appeared consistently in one-summer common carp in July and August and in two-summer carp from March to the end of May. These cysts were localized strictly at the base of the gill filaments or in the gill arch and were surrounded by a capsule of connective tissue (Kovács-Gayer & Molnár, 1983).

M. dispar was also of common occurrence, observed first in 4 to 5-month-old fish, in which it formed oval cysts, the size of a pinhead, at the apical and medial part of the gill filaments of common carp fry. A total of 12% of overwintered common carp fry showed infection of the gill filaments by *M. dispar* in the period between April and August.

DISCUSSION

The present results represent a fundamental shift from the previously held views on the development of *M. cyprini*, and render possible a realistic assessment of the pathogenic role of this species. Contrary to views accepted in the literature, according to which *M. cyprini* develops in smaller plasmodia in various tissues of the fish, our studies have proved that *M. cyprini* is a parasite of striated muscle. With *M. cyprini*,

the entire development, including spore formation, takes place in the skeletal muscle fibres. Thousands of spores develop in the enormously swollen muscle fibres and, after disintegration of the cells, spores are released into the intermuscular connective tissue. Most spores gain entry to the blood stream, possibly via the lymphatic system. Spores reaching various organs are retained in small capilaries. Their further fate depends on their location: those located in the skin and gills are released directly and some from intestine, liver and kidney reach the external environment via the intestinal lumen, kidney or bile ducts in 'yellow bodies' representing spores encapsulated by host cells. Spores without direct access to the exterior are destroyed by encapsulation by epitheloid cells, then connective tissue cells if they are retained in the muscle after release from the pseudocysts or by macrophages if transported to the viscera.

After some pseudocysts have liberated spores, others continue to develop so that scattered spores and pseudocysts are found simultaneously in the same fish.

The host specificity of *Myxobolus* spp. is not known and it is not clear whether the parasites described as *M. cyprini* in different fish really belong to this species. It is possible that sometimes the parasites identified as *M. musculi* are in fact the muscle stages of *M. cyprini*. These questions can only be settled by experimental infections, but transmission mechanisms have not yet been fully elucidated. Species of *Myxobolus* known from each fish species, particularly those in the common carp, can be determined on spore morphology and location within the host organism.

The majority of our studies indicate that, in most cases, infection with *M. cyprini* runs a latent course in which the developing cysts cause subclinical damage to the muscles, and the spores, which are scattered throughout the body, result only in local necroses. However, heavy infections, which were observed in one fish pond, were accompanied by clinical and histopathological signs corresponding to a pathological picture similar to malignant anaemia, a disease characterized by hydropic degeneration and dropsy. In these heavy infections there were large numbers of spores distributed to the capillaries from the muscles and these were thought to cause microtraumas by obstructing blood circulation which may have led to the hydropic changes and haemorrhages.

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