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Myxosporean vegetative stages in the choroidal *rete mirabile* of *Gasterosteus aculeatus* infected with *Myxobilatus gasterostei* and *Sphaerospora elegans*

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ABSTRACT: Examination of samples of three-spined sticklebacks *Gasterosteus aculeatus* from Loch Flemington in Scotland and from 3 sites in southern England revealed hitherto unknown myxosporean stages in the choroidal *rete mirabile* of the eye. This paper describes the light and ultrastructural features of these stages and discusses affinities with PKX and extrasporogonic stages of other myxosporean parasites, particularly those belonging to the genus *Sphaerospora*.

INTRODUCTION

Since the discovery (Csaba 1976) and correct interpretation (Bucsek & Csaba 1981, Lom et al. 1983, Csaba et al. 1984) of the proliferative blood-stream stages of *Sphaerospora renicola* Dyková & Lom, 1982, similar blood-stream stages have been found in 19 other species of the genus *Sphaerospora* (Lom et al. 1985, Baska & Molnar 1988, Hedrick et al. 1988, Kepr & Trsová 1989). Extrasporogonic stages of *S. renicola* were also found in other sites in the swimbladder (Kovacs-Gayer et al. 1982, Körting 1982), and in the epithelial cells of renal tubules (Lom & Dyková 1985). Similar extrasporogonic stages have been found in *Myxidium lieberkuehni* (Lom et al. 1989). Their existence may therefore be widespread in *Sphaerospora* and other myxosporean genera.

An investigation of myxosporidiosis in the three-spined stickleback *Gasterosteus aculeatus* initially revealed hitherto unknown myxosporean stages in the capillaries of the choroidal *rete mirabile* ('choroidal gland') of the eye, and subsequently also in the swim-bladder *rete*. This paper presents the results of the study, and discusses affinities with PKX and extra-sporogonic stages of other myxosporean parasites.

MATERIAL AND METHODS

Sticklebacks were collected from Loch Flemington, Scotland (57° 32' N, 3° 58' W), and from sites in the

Table 1. *Gasterosteus aculeatus*. Examination of sticklebacks from the south of England for choroidal *rete* vegetative stages of myxosporeans

Source	No. examined	% Prevalence *Sphaerospora elegans*	% Prevalence *Myxobilatus gasterostei*	Choroidal *rete* stages
R. Avon	17	100	100	29
R. Weaver	50	40	0	0
R. Hooke	15	35	6.6	6.6

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south of England, namely the River Avon (Wiltshire) (51° 01' N, 01° 45' W), River Weaver (Devon) (50° 51' N, 03° 19' W) and River Hooke (Dorset) (50° 48' N, 02° 39' W). Fish were maintained in laboratory aquaria with a constant supply of aerated and dechlorinated water. Fish were autopsied soon after return to the laboratory and at intervals thereafter. Tissues were fixed in 10% neutral buffered formalin and routinely processed to paraffin wax or resin sections.

Smear preparations of the choroidal *rete* were stained with Giemsa and samples of the *rete* were prepared for electron microscopy. They were fixed in 2% glutaraldehyde in 0.1 M cacodylate buffer, post-fixed in 2% osmic acid in the same buffer and embedded in Spurr resin. Ultrathin sections were stained with uranyl acetate and lead citrate and examined with a Philips 301 electron microscope at 80 kV accelerating voltage.

RESULTS

The sticklebacks from Loch Flemington were heavily infected with a variety of protozoan parasites: *Glugea anomala* (prevalence 25%), *Goussia gasterostei* (40%), *Myxobilatus gasterostei* (100%), and *Sphaerospora elegans* (83%). Myxosporean vegetative stages in the capillaries of the choroidal *rete* were detected in fresh mounts in 25% of the sample; the intensity of infection was sometimes high, so that they could easily be found. The stages were seen, in a small number of cases, in the swimbladder *rete mirabile*. Data from fish from sites in England are given in Table 1.

When examining a fresh mount, the stages could be distinguished as round or ellipsoidal bodies (Fig. 1) lying in the capillaries of the *rete*. They were 8 to 22 µm in diameter and appeared to have structureless peripheral cytoplasm containing several inner bodies and a few refractile granules. They were non-motile, seeming to adhere quite firmly to the surface of the endothelial cells so that they are not dislodged when preparing a smear. This is probably why they are difficult to detect in Giemsa-stained impression smears.

Ultrastructurally, the choroidal *rete* stages ranged from small spherical primary cells with one secondary cell which left enough space (Fig. 2) or insufficient space (Fig. 3) for the red blood corpuscles to pass by, to large primary cells with numerous internal cells which completely occluded the capillary possibly preventing blood circulation through that capillary (Fig. 4). Most of the sectioned primary cells included 1 to 5 secondary cells; exceptionally, 9 or 10 and once even 18 secondary cells could be seen in the section. In some primary cells, one of the secondary cells, but not more, harboured 1 or 2 tertiary cells. Tertiary cells could be seen even in primary cells with just 1 secondary cell. Although counts of the number of cells contained within 1 primary cell cannot be exact from thin sections, they nevertheless give some indication of the approximate structure of the primary cell.

The primary cells adhered to the endothelial cell membranes leaving gaps of 35 to 50 nm (Fig. 5). Sometimes the gap was wide and filled by a moderately opaque substance (Fig. 5). There were no hemidesmosome-like junctional complexes on either side of the host-parasite boundary. The primary cells sometimes had surface projections, interlocking with the endothelial cell surface.

The primary cells had a thin, finely granular, homogeneous ectoplasmic layer, and their cytoplasm contained various vesicles of different sizes, mostly vacuole-like (Figs. 7 & 8) sometimes with more or less electron-opaque amorphous substance, mitochondria, one to several Golgi, sparse cisternae of endoplasmic reticulum and some free ribosomes.

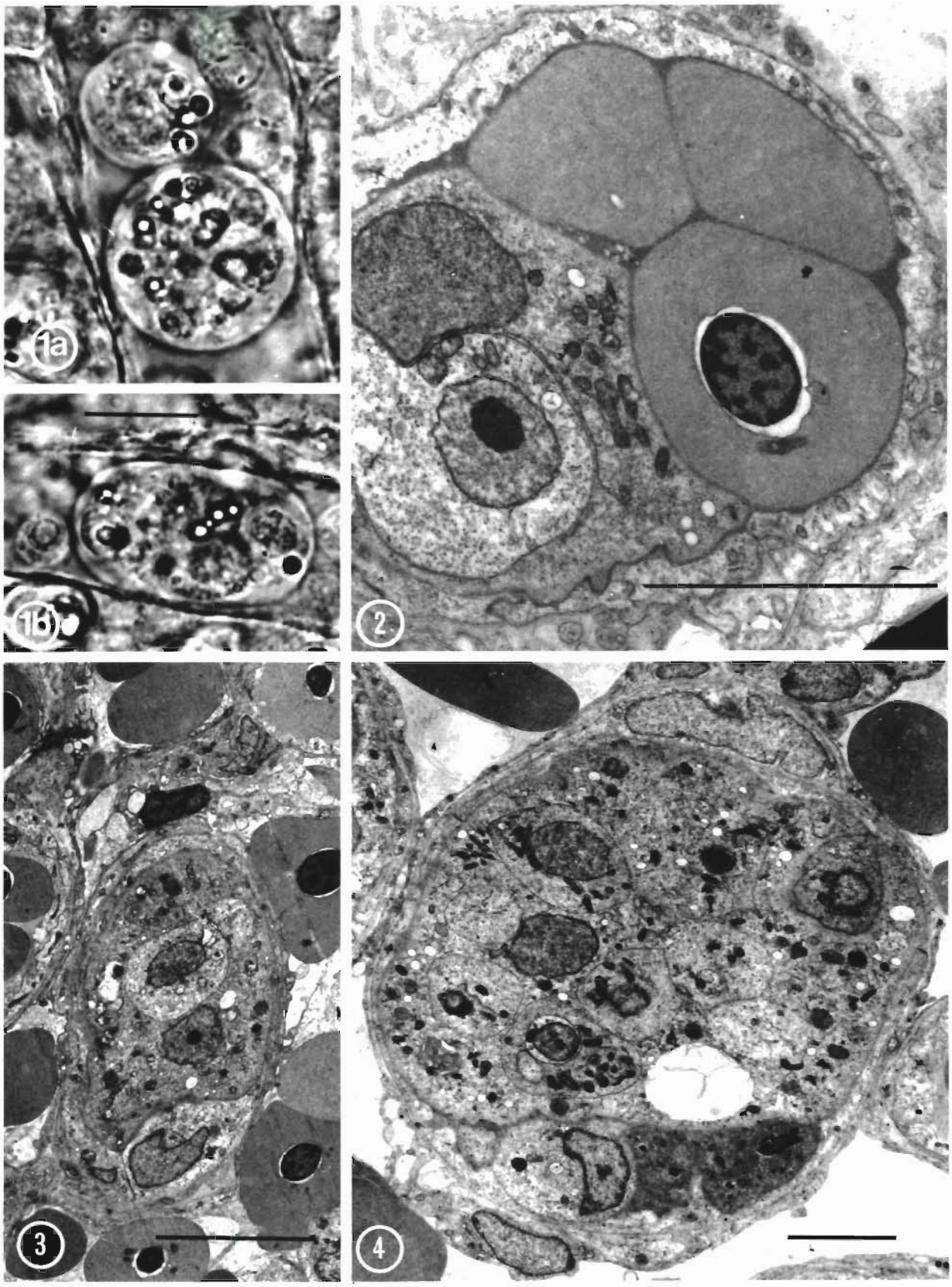
Secondary cells seen in section ranged from presumably young ones, with a small volume of lucent endoplasm, a few mitochondria and vesicles, to presumably mature ones, with denser cytoplasm and a variety of cytoplasmic organelles including typical Golgi, bundles of microtubules which were either typically straight (Fig. 6) or arched, and sometimes with plentiful free ribosomes and other inclusions. The contents of all cell inclusions may vary depending on the stage of secondary cell development. The cell surface may be raised into short projections. Secondary cells were either isolated in the primary cell cytoplasm, or lay together, in groups of 2 to 5, in a common membrane, indicating previous divisions within this 'vacuole'. In some secondary cells, there were 2 nuclei or a possibility of a forming partition (Fig. 8), representing secondary cell division.

Tertiary cells always resembled young secondary cells. Most of their volume was occupied by a nucleus leaving a thin layer of electron-lucent cytoplasm beneath the cell membrane. The cytoplasm only contained 1 mitochondrion in the section and no other organelles (Fig. 7).

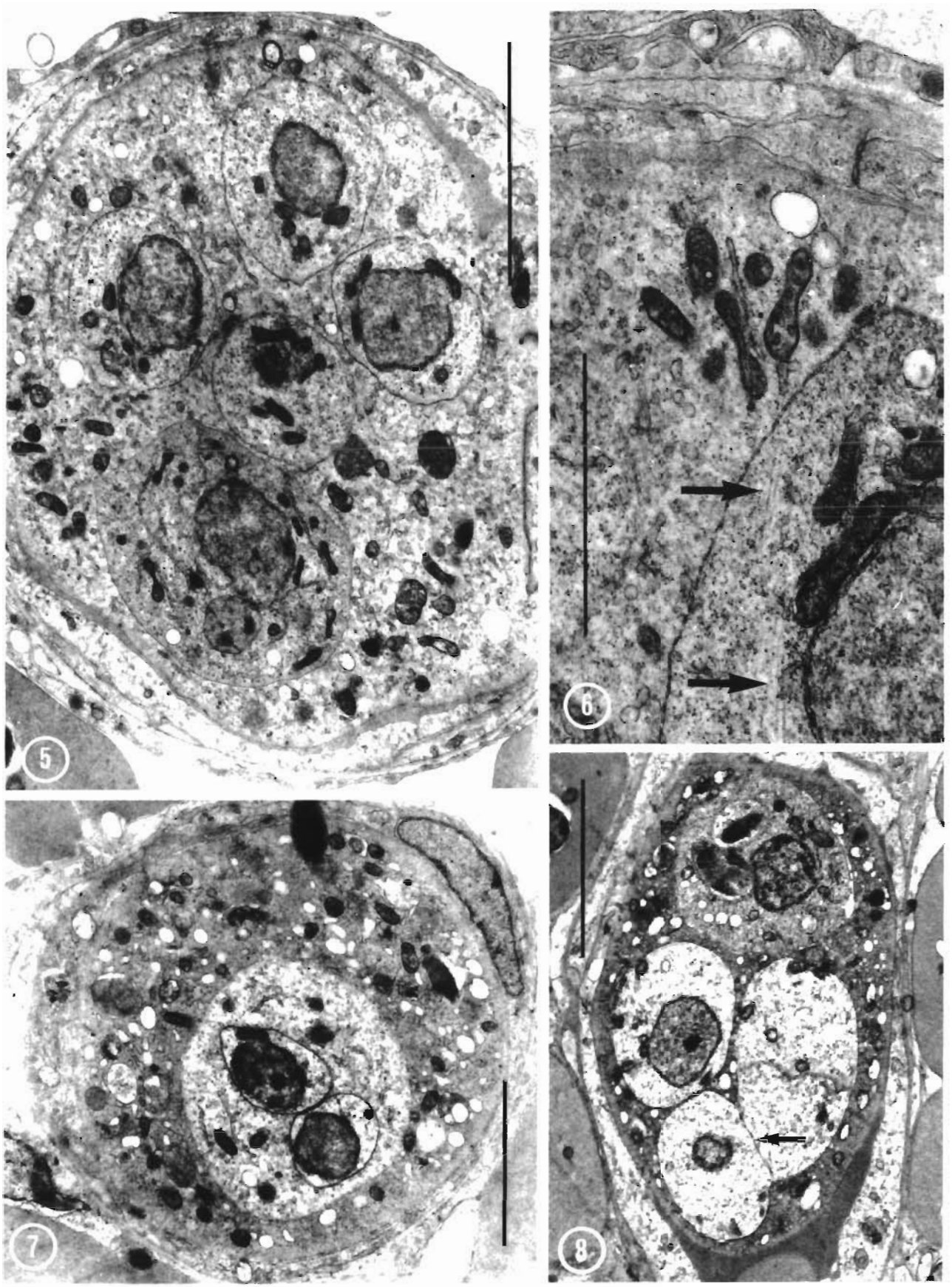
In many primary cells, empty spaces containing an unstructured mass of cytoplasm indicated where one of the secondary cells had died.

DISCUSSION

The organism from the choroidal or swimbladder *rete* certainly represents a stage in an extrasporogonic sequence of a myxosporean developmental cycle. An extra-sporogonic stage may be defined (Lom 1987) as one that proliferates, without spore formation, prior to



Figs. 1 to 4. Myxosporean vegetative stages in *Gasterosteus aculeatus*. Fig. 1 (a),(b). Choroidal *rete mirabile* stages in fresh mounts of the *rete* capillaries. Bar = 10 μm. Fig. 2. Small stage within the capillary, showing the nucleus of the primary cell and one secondary cell. Bar = 5 μm. Fig. 3. More advanced stage completely occluding the *rete* capillary. Bar = 10 μm. Fig. 4. Large stage with many secondary cells within a *rete* capillary. Bar = 5 μm



Figs 5 to 8 Myxosporean vegetative stage in *Gasterosteus aculeatus* Fig. 5. Primary cell with 5 secondary ones; an opaque substance fills the narrow space between the endothelial cell and the surface of the parasite. Bar = 5 µm. Fig. 6. Microtubules in the cytoplasm of the secondary cell Bar = 2 µm. Fig. 7. A primary cell with 1 secondary cell containing 2 small tertiary cells. Bar = 5 µm Fig. 8. A structure (arrowed) suggesting a developing partition between 2 dividing secondary cells. Bar = 5 µm

or parallel with the sporogonic phase and at a site other than that in which sporogony takes place. It obviously belongs to the developmental cycle of one of the 2 myxosporeans found in the sticklebacks. Thus far, samples in which only one species is present have been few in number (Table 1) and since choroidal *rete* stages are not present in all infected individuals it is not possible to be sure to which species they belong. No extrasporogonic stages of the genus *Myxobilatus* have been reported – Molnar (1988) reported only early intracellular trophozoites of *M. legeri* in epithelial cells of the cyprinid renal tubules in which the sporogonic phase took place – but such a possibility in any myxosporean cannot be excluded. Thus unusual early stages of infection with *Myxobolus cerebralis* were found in subdermal tissues of rainbow trout (Daniels et al. 1976, Markiw 1989). However, *Sphaerospora* spores have been known, as mentioned in the introduction, to have various extrasporogonic stages. Therefore we feel entitled to assign tentatively our findings to the developmental cycle of *S. elegans* unless future experimental work proves otherwise.

The choroidal and swimbladder *rete* stages do represent a category different from the known types of extrasporogonic stages of *Sphaerospora* species. They differ from the bloodstream stages in being immobile and in possibly having a predilection for the choroidal *rete*; they were not often found in the swimbladder *rete*. Our limited observations in this respect (Lom unpubl.) show no preference of *S. renicola* bloodstream stages for the choroidal *rete* in carp. They differ from intracellular stages of *S. renicola* forming nodules in renal tubules of carp (Lom & Dyková 1985) in not exceeding the level of tertiary cells. There are no essential differences, however, in the cell structure of the *rete* stages on one side and the bloodstream stages (Lom et al. 1983) and swimbladder stages (Dyková et al. 1990) of *S. renicola* on the other side. Meticulous observation may reveal somewhat smaller quantities of free ribosomes and glycogen granules in *rete* stages while the latter have more cytoplasmic vacuoles and vesicles. The curious fact that no more than one secondary cell on a section contained a tertiary cell does not necessarily mean that there were no more, since serial sections were not observed. However, in this respect the *rete* stages also differ from bloodstream and swimbladder stages of *S. renicola*. *Rete* stages comply with the common organisation of early developmental stages of Myxosporea.

We assume that after they have left the *rete*, the stages reach the kidney to transform into sporogonic pseudoplasmodia. Supposing that the *rete* stages do belong to the life cycle of *Sphaerospora elegans*, the observations on their cell structure do not lend any support to, but neither do they contradict, the assumption (Kent & Hedrick 1986, Feist 1988) that this species may be (one of?) the organism(s) with which PKX, the still enigmatic protozoan agent of proliferative kidney disease of salmonids, is identical.

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