[Jpn. J. Parasitol., Vol. 39, No. 2, 229–233, April, 1990]

Research Note

A Flagellate Isolated from the Urine of a Woman

TADASUKE ONO[1)*], TOSHIO NAKABAYASHI[1)**], TOSHISIGE TANAKA[2)], MASAHIDE FUJITA[2)], TETSUO TAGUCHI[2)], TAKAKO MORITA[3)] AND TERUO KITANI[3)]

(Accepted for publication; April 6, 1990)

Key words: Flagellate in urine, *Bodo* sp.

Some flagellates besides *Trichomonas vaginalis* have been found in human urine up to the present time (Otsuru, 1985). But the flagellates have not been observed twice or more in succession in the urine of the same person. Furthermore, the flagellates have been found in the urine of both patients and healthy persons. Therefore, further evidence is necessary to confirm whether the flagellates in urine are really parasitic or not. The present paper reports on a case of human cystitis in which the flagellates were found continuously in urine for 11 consecutive days, and includes the morphological features of the flagellates.

The patient was a 65-year old female housekeeper living in Ibaraki City, Osaka Prefecture, Japan. She was admitted to the hospital attached to the Research Institute for Microbial Diseases, Osaka University, on June 1, 1986, because of abdominal pain, liver deficiency, cholangitis and cystitis. The patient underwent choledochotomy and papilloplasty of duodenum on July 1, 1986. From the beginning of her hospitalization, microscopical examinations of urine showed intermittent contamination with bacteria, yeasts, demarcated epithelial cells, erythrocytes and neutrophilic leucocytes. On July 14, 1986, we observed a large number of flagellates along with other microorganisms and blood in a urine specimen (Fig. 1). The flagellates were observed for 11 consecutive days on and after July 14. It is possible that the flagellates had been discharged in the urine for longer than 11 days, but this is not known for certain because a urine examination was not performed for several days prior to July 14. The parasites were present in considerable number in the urine and showed very active motility. The number of flagellates calculated on July 22 and for 3 successive days was in the range from 250 to 1,000/ml of urine. The flagellates remained alive in the urine for 24 hrs at room temperature. The flagellates were not found in the woman's stool specimens with direct smears. Then, the in vitro culture of the stool specimens was attempted with Ringer solution containing 12.5% horse serum, 0.85% saline solution and Tanabe-Chiba medium at 30°C and 37°C. After cultivating them for 1 week, the same flagellates as detected in the urine were observed only in the 0.85% saline solution,

[1)]*Department of Protozoology,* [2)]*Department of Surgery and* [3)]*Department of Internal Medicine, Research Institute for Microbial Diseases, Osaka University, Suita, Osaka 565 Japan*

*To whom correspondence should be addressed

Present address: *Department of Veterinary Epidemiology, College of Agriculture, University of Osaka Prefecture, Mozu-Umemachi, Sakai, Osaka 591 Japan

**Department of Parasitology, School of Medicine, Fujita-Gakuen Health University, Toyoake, Aichi-ken 470-11 Japan

This work was presented at the 24th West Japan Regional Meeting of Parasitology in Nishinomiya City, October 11-12, 1986

小野忠相　中林敏夫（大阪大学微生物病研究所原虫学部門）
田中利茂　藤田昌英　田口鐵夫（同、外科学部門）
森田隆子　木谷照夫（同、内科学部門）

at 30°C.

The flagellates isolated from the urine were cultivated in RPMI 1640 medium and DMEM at 30°C under the cohabitation of two species of *Pseudomonas, P.putida* and *P.maltophilia.* The flagellates were found to grow luxuriantly in all these media with or without 5% horse serum and to survive in them for at least three months without an exchange of medium. The flagellates did not grow when more than 10% horse serum was added to the same medium. All attempts to multiply the flagellates under bacteria-free conditions in the medium were unsuccessful.

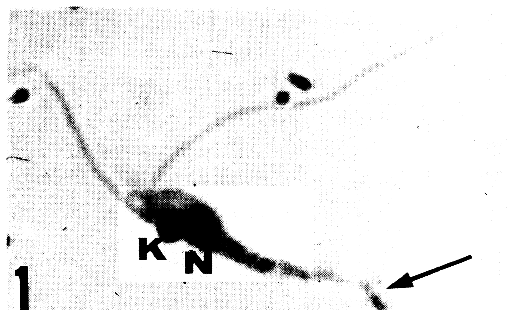
For light microscopical observations, the parasites cultivated in RPMI 1640 medium were smeared on microscopical slides, fixed in absolute methanol and stained with Giemsa's solution diluted with 1/30 M phosphate buffer, pH 7.2. For scanning electron microscopical observations, the flagellates cultivated in RPMI 1640 medium for 10 days were collected by centrifugation at 3,000 rpm for 10 min and the sediment was fixed at 4°C for 1 hr in 0.01 M phosphate buffer (pH 7.4) containing 2.5% glutaraldehyde, washed for 1 hr with phosphate buffer containing 0.25 M sucrose and post-fixed at 4°C for 1 hr with 1.5% osmium tetraoxide in isotonic buffer. The fixed sample was dehydrated in absolute ethanol, treated with isoamyl acetate, and dried in a critical point drying apparatus. After gold palladium coating, the specimen was observed with a Hitachi HSM 2A scanning electron microscope. For transmission electron microscopic observations, after post-fixed with 1.5% osmium tetraoxide, the sample was dehydrated in water-ethanol mixture and absolute ethanol, the pellet was treated with propylen oxide and embedded in epoxy resin. The sections were double stained with uranyl acetate and lead citrate.

In Figs. 1 and 2, the flagellate is ovoidal, $6.4 \pm 0.2\ \mu m \times 2.4 \pm 0.2\ \mu m$ in size, with a broad anterior end. It is remarkable that an elongate mass is attached to the tapering posterior end (arrows in Figs. 1 and 2). The flagellate has two free flagella, anterior and posterior, and a nucleus and a kinetoplast. A small depression and a beak-like process are observed at the anterior end. The posterior flagellum, which is usually more than twice as long as the anterior one, runs backwards. The nucleus is located in the anterior region of the cytoplasm. A Feulgen-positive kinetoplast is located between the nucleus and the kinetosomes of the flagella. The kinetoplast is a large round body appearing more compact and densely stained than the nucleus. Figure 3 shows two flagella arising from the flagellar pocket (arrow). Figure 4 shows the kinetoplast and the nucleus which lie very closely. The kinetoplast envelope can not be seen clearly in this figure. There are many glycogen vacuoles in the cytoplasm. The pellicular microtubules are observed in a single layer array below the surface membrane. The marginal extension of the flagellar pocket is prominent. Figure 5 shows the flagellar pocket and the buccal cavity. The axoneme and the paraxial rod (arrow) are found in the flagella located in the flagellar pocket. Figure 6 shows the large kinetoplast-mitochondrion complex surrounded by the two-layered kinetoplast envelope. The

Fig. 1. A light micrograph of the flagellate shows two flagella, anterior and posterior, and both a nucleus (N) and a kinetoplast (K). An elongate mass of the cytoplasm (arrow) is attached to the tapering posterior end. ×7,000

Figs. 2–3. Scanning electron micrographs of the flagellate. Fig. 2 shows the broad anterior end of the flagellate and a tapering posterior end which sticks an elongate mass (arrow). ×12,000. Fig. 3 shows two flagellar arising from a flagellar pocket (arrow) near the anterior end of the flagellar. ×24,000

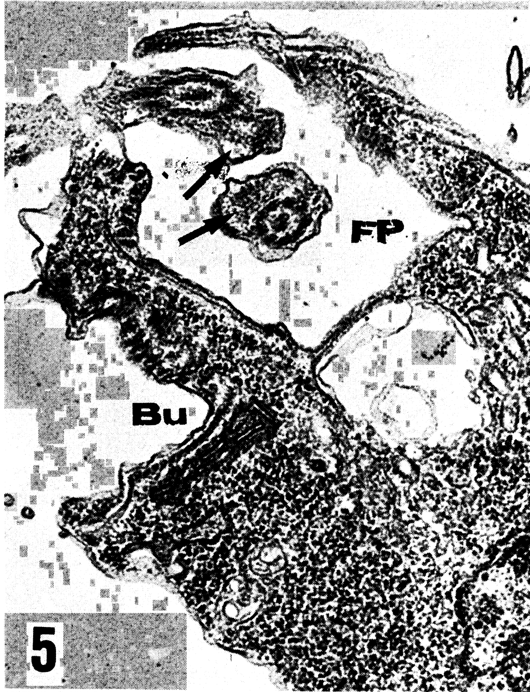
Figs. 4–7. Transmission electron micrographs of the flagellate. ×37,000. In Fig. 4, a longitudinal section of the flagellate shows the kinetoplast (K) and the nucleus (N) laying very closely in the cytoplasm. In Fig. 5, a transverse section shows the relationship between the buccal cavity (BU) and the flagellar pocket (FP). Two flagella, with each having a paraxial rod (arrow), can be seen in the flagellar pocket. Fig. 6 shows the kinetoplast which appears as a cluster of numerous fine filaments. A large kinetoplast-mitochondrion complex surrounded by a two-layered kinetoplast envelope occupies the major part of the cytoplasm. The matrix of the mitochondrion with cristae is seen around kinetoplast. No pellicular microtubules can be found below the surface membrane in this sectional plane. Fig. 7 shows the posterior half of the flagellate which is occupied by various sizes of food vacuoles. The food vacuoles contain many bacteria in varying stages of breakdown. The undigestable materials are excreted by the coalescence (arrow) of the cell plasma membrane and the food vacuole.


K
N
1

2

3

4


FP
Bu
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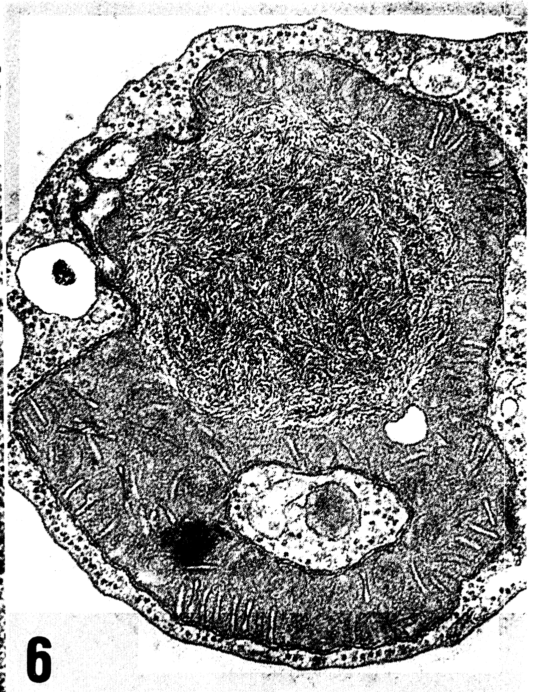
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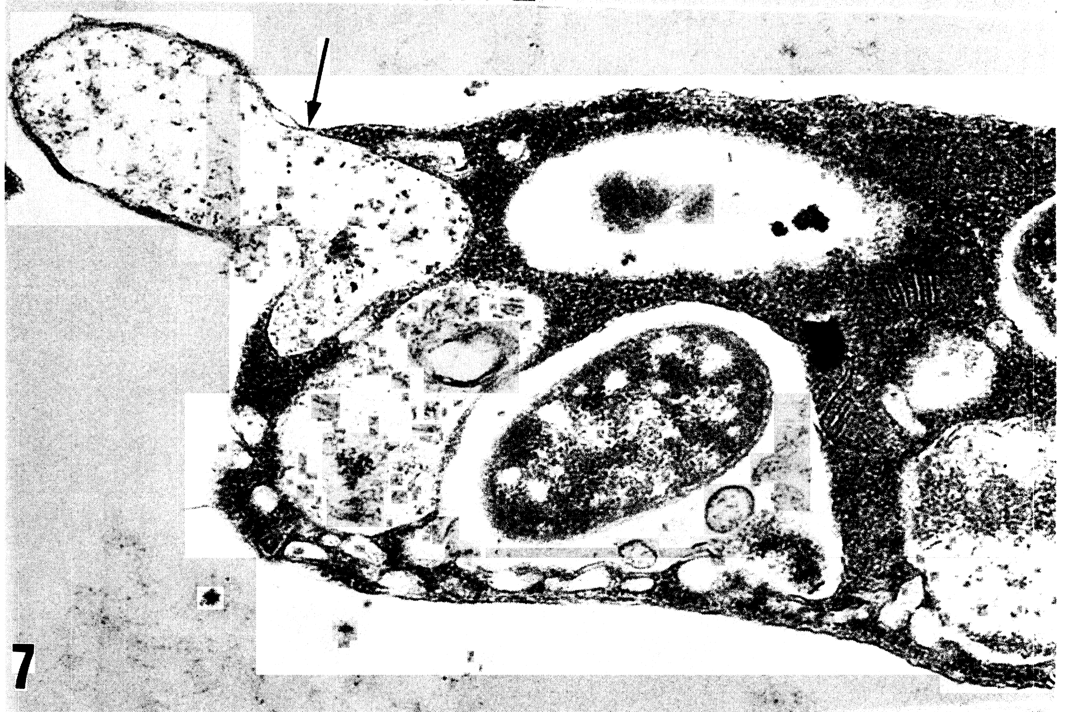
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kinetoplast looks like a cluster of numerous fine filaments and is surrounded by the mitochondrion. No pellicular microtubules is seen below the surface membrane. Figure 7 shows the posterior half of the cytoplasm where many food vacuoles accumulate. Many bacteria in various degenerative stages in still remain in the vacuoles. The food particles ingested through the buccal cavity are digested in the food vacuoles. Then, the vacuoles containing digested foods migrate backwards in the cytoplasm and then, are excreted through posterior end of the cytoplasm. The coalescence of the cell membrane and the food vacuole membrane (arrow) indicates a stage in the excretion process.

Kawamura (unpublished paper, 1975) found that a species of flagellate was discharged for 3 weeks in the urine of a patient with urinary disorder. It was different from the *Trichomonas* species. By an examination with a ureteral catheter, he supposed that the flagellates inhabited in the kidney of the patient. Otsuru (1985) detected a species of flagellate in the urine of a patient of psychosis and suggested that the flagellate belonged to Genus *Bodo* based on light microscopical observation. Nagase (personal communication, 1987) found the same species of flagellate in the urine examined for a person's health examination as that was reported in the present paper. In their cases except for Kawamura's case, however, patient did not show any trouble in urinary organ and the flagellates were found only once in the urine. In the present case, from the beginning of hospitalization the patient discharged intermittently bacteria, yeasts, many demarcated epithelial cells, erythrocytes and neutrophile leucocytes in the urine and had showed discharge of flagellates into urine continuously for 11 consecutive days after the 44th of hospitalization. Therefore, it is unlikely that flagellate-infection in urinary organ resulted in cystitis. But, the patient was healed of her cystitis without any treatment after that time when discharge of the flagellate into urine had been stopped. It may mean that the flagellates to some extent participate in cystitis.

Since the flagellate could be isolated from the stool of the patient, it is presumable that the flagellate urinary infection was transferred from the pre-existing intestinal infection. The optimal temperature for growth of the flagellates in RPMI 1640 medium and DMEM was 30°C. The flagellate could grow in in vitro culture at 34°C, but died within 24 hrs at 37°C. It is difficult to explain why the flagellates isolated from human urine showed the maximum growth at 30°C, and died within 24 hrs at 37°C in the in vitro culture condition. This may be the result of the change in temperature sensitivity to the growth of the flagellates or other factors, such as nutritional conditions and the growth of the concomitant bacteria in the cultures. Further study to solve this question will be necessary.

The flagellates isolated from the present patient were characterized ultrastructurally by the presence of a large kinetoplast-mitochondrion complex, two flagella arising from a flagellar pocket and a paraxial rod associated with the axoneme of each flagellum. These ultrastructural features corresponded to a flagellate species belonging to the Genus *Bodo* (Pitelka, 1961; Brooker, 1971; Burzell, 1975). Sinton (1912) found a species of flagellate in the urine of the patient suffered from malignant tertian malaria, and named the flagellate *Bodo urinarius.* However, the flagellate in the present paper could not be identified immediately with this species of *Bodo*, because Sinton's description was based on light microscopical observation.

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