Cytoplasmic Vibrations Due to Flagellar Beating in *Trichoplax adhaerens* F. E. Schulze (Placozoa)*

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The dorsal epithelium of the plate-like primitive metazoon *Trichoplax adhaerens* is a sheet of flat cells, each bearing a single flagellum. This layer rests largely on an intercellular fluid and is predominantly fixed at the edges of the organism but also locally by fibre cells constituting the middle layer of the animal. Within these extensile dorsal cells the counterforce of the flagellar power stroke acting on the basal bodies subjects nearby objects to oscillatory motions. Given certain mechanical conditions, the vibrating single basal body can be compared with a springy oarlock that is rhythmically displaced with the power stroke and recoils during the recovery stroke. The basal body transmits its motion to the adjacent viscoelastic cytoplasm in which naturally coloured inclusion bodies serve as markers of the pulsating deformation visible in the living animal. These inclusions vibrate in the frequency range of the flagellar beat and stop as soon as the flagella are paralyzed by chemical means. That the vibrations can only intermittently be observed may be due to the varying tension of the dorsal epithelium exposed to the forces of the contractile fibre cells. – The rhythmic cytoplasmic deformation synchronous with the flagellar action has apparently not yet been described in metazoa and could be documented by video recording.

Introduction

"Surprisingly little is known about the mechanical factors that determine ... the organelle movement ... of cells" (Valberg [1]). This recent statement implies that any newly observed phenomenon of intracellular motion should be thoroughly analyzed and described. While studying the collection of food particles by the dorsal flagella of *Trichoplax,* I became aware of a rhythmic displacement of inclusion bodies in the superficial layer. As these movements appeared to be synchronous with the flagellar beat, I decided to more closely investigate this occurrence since reports of passive cytoplasmic responses to flagellar activity in Protozoa are scanty and in Metazoa seem to be totally lacking.

Trichoplax adhaerens (F. E. Schulze, 1883) is a most primitive plate-like metazoan (diameter 0.2–3 mm), flagellated all over and living in the coastal areas of tropical and subtropical seas. The animal's body is composed of a dorsal and a ventral epithelium of flat and cylindrical cells respectively and an intermediate layer of contractile "fibre cells" with long extensions. The morphology of *Trichoplax,* the only representative of the phylum Placozoa, has been described in detail by Grell and coworkers [2–5] and Ruthmann and coworkers [6–9]. The characteristic adhesion of the animal to the substratum is probably mediated by small cytoplasmic laminae protruding from the ventral cells, but surface tension plays a role when the animal adheres upside down to the surface of the culture medium. – *Trichoplax* feeds predominantly on unicellular organisms, mostly algae. When the animal passes over algae covering the substratum, enzymes from the ventral epithelium digest the plant cells extracellularly; but, besides "grazing", food particles are gathered on the dorsal surface by directed flagellar action, often followed by cytophagy (Wenderoth [10]). – While moving on the ground the animal constantly varies its outlines but alters its site on the substratum only slowly. Reproduction is usually asexual by simple fission or by detachment of swarmers [11], but egg production and development of the ovum up to 16 cells has also been observed [12], so-called S-cells possibly representing sperms.

* A videotape showing the motion phenomenon described may be requested as a loan from the author.

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Materials and Methods

The animals used in this study came from the Red Sea and have been cultured for years in our

department. *Trichoplax* specimens are kept in plastic dishes with artificial sea water. The animals feed on unicellular algae of the genus *Cryptomonas* (Cryptophyceae). *Trichoplax* individuals were taken out of the culture medium by pipette aspiration and either examined alive or prepared for TEM. The observation chamber for light microscopy was 150 µm deep, filled with sea water and mounted on a microscope slide, with coverslips as spacers. The animals, 20–30 µm thick, settled on the slide and could move freely in the chamber. After studying the motion of the inclusion bodies and recording their vibrations on video tape, a few *Trichoplax* individuals were transferred to a hypotonic medium (2.1 instead of 3.3% salt concentration) to osmotically increase the intercellular fluid space. By this treatment, the dorsal epithelium is largely detached from its connections to the middle layer of fibre cells and the spreading of the vibrations over the whole surface area can be observed. The animals tolerate this change in environment for more thank a week. – With one exception (Fig. 1), the specimens were viewed and photographed with 40 × and 100 × apochromate objectives equipped with phase contrast optics. An inverted microscope permitted inspecting of the ventral epithelium. A video camera (Panasonic F 10 CCD) with monitor transferred the microscope images of the motion phenomena simultaneously to a tape recorder with real time registration. A series of photographs was taken from the display screen for obtaining time-lapse pictures of the particle motions. High-speed cinematography with Nomarski interference contrast optics was used to count the heating frequency of the flagella. A 12 nmol solution of sodium orthovanadate in sea water as a dynein inhibitor was added during microscopic observation in order to follow the slowing down and final stopping of the flagellar movements. Giemsa-stained squash preparations were made as described previously [13]. Whole *Trichoplax* were fixed in a mixture of 1% OsO_4 and 2.5% glutaraldehyde, embedded in Epon, and cut with glass knives on a Reichert OM U2 microtome. The sections, either parallel or perpendicular to the dorsal surface of the animal, were poststained with uranyl acetate and lead nitrate and viewed with a Philips EM 410 electron microscope.

Results

Size, outline, and surface appearance of a *Trichoplax* individual are shown in Fig. 1. While the living animal moves incessantly in a gliding manner its outlines change, but hardly its site on the substratum. Inclusion bodies, named "concrement vacuoles" by Grell [2], appear in the light microscope as dark spherical corpuscles of 1–2 µm (Fig. 2). There is only a single particle per cell though larger aggregates of included material occur (Fig. 5). The natural reddish colour of the inclusions gives the whole animal a crimson tinge particularly visible, even with the naked eye, in spontaneously contracted specimens detached from the substratum. Giemsa-stained squash preparations demonstrate that the inclusion bodies are strongly basophilic and are present in approximately half of the cells and that they are randomly distributed in the cytoplasm (Fig. 9).

Fig. 1. *Trichoplax adhaerens* in culture dish, viewed from above. Flagella-collected unicellular *Cryptomonas* algae in large numbers on the dorsal surface. Bar = 1 mm.

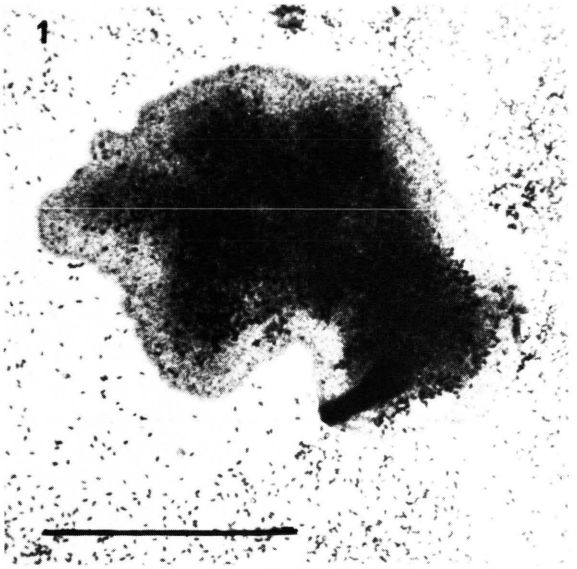
Fig. 2. Live *Trichoplax*, unstained, viewed from above, bright field, transmitted light. Photograph taken from the video screen. Dark inclusions in the superficial cells. Bar = 10 µm.

Fig. 3. Flagella at the edge of the living animal. Photograph taken from a single picture of a 16 mm high-speed cinematographic film. Nomarski interference contrast optics. Bar = 5 µm.

Fig. 4. Perpendicular section of *Trichoplax* near its edge, TEM photograph. Flat dorsal epithelium (D) lying mostly on the intercellular fluid. The tension of the dorsal layer is not evident in the specimen detached from the substratum. Some inclusions marked by arrow heads. Bar = 10 µm.

Fig. 5. Perpendicular section of *Trichoplax* with single and aggregated intracellular inclusions. Bar = 5 µm.

Fig. 6. Perpendicular section. Two inclusions containing the typical osmiophilic, coarsely granulated material, included in separate membranes, lying in dorsal cell extensions directed downwards; one cell connects the middle layer. Bar = 1 µm.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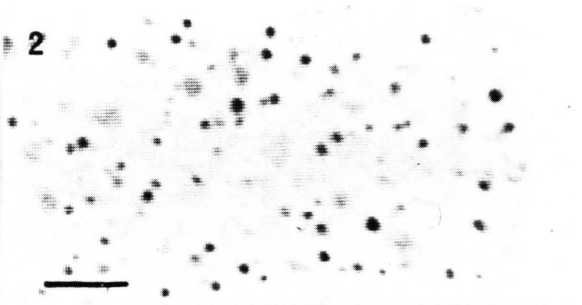
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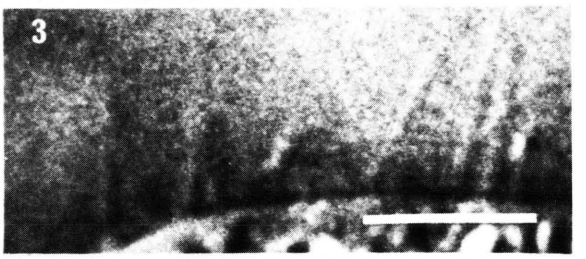
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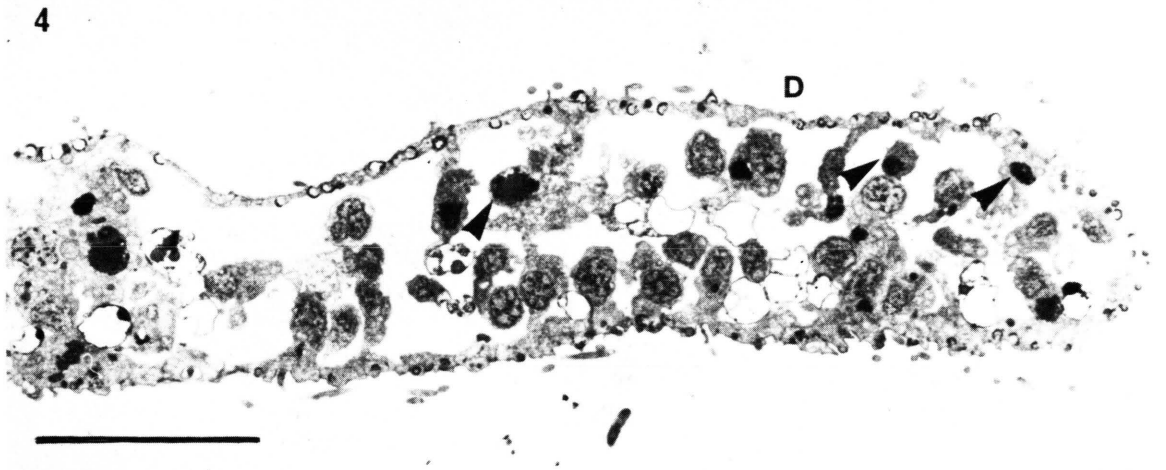
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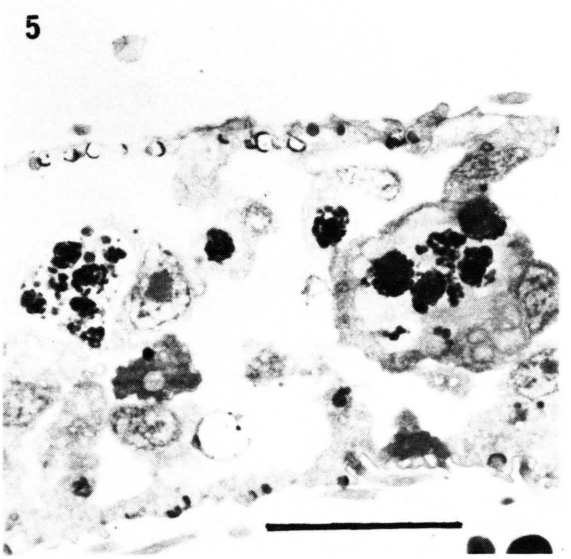
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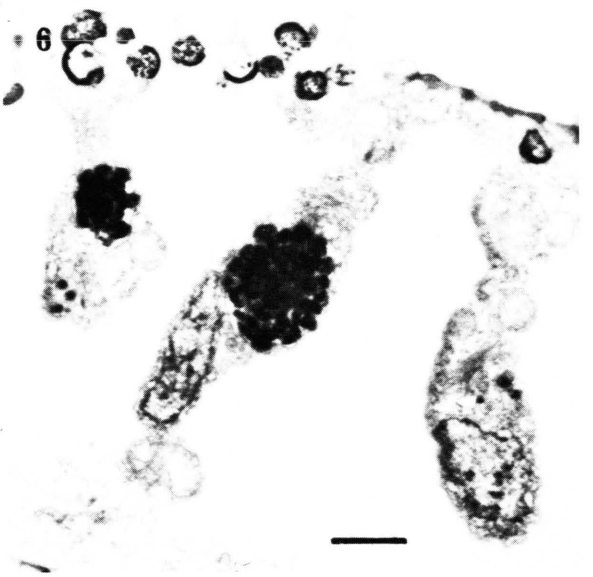
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The general arrangement of the *Trichoplax* tissues as viewed in TEM is shown in a perpendicular and radial section (Fig. 4). While the ventral epithelium is composed of cylindrical cells grouped in a palisade-like manner the dorsal epithelium is mostly flat and rests largely on the intercellular fluid. Vertical portions of the dorsal cells are directed downwards and contain the nuclei as well as the inclusion bodies that were already seen with the light microscope. These cell organelles consist of dense accumulations of an osmiophilic, coarsely granular substance that cannot further be differentiated at higher magnifications (Fig. 5–7). These inclusions are usually surrounded by a delicate membrane and are unmistakably different from the finely granular vesicles that are situated in the flat portions of the dorsal cells (see Fig. 7). The ventral extensions of the dorsal cells are often fixed to the fibre cells of the middle layer. Thus, a fluid-filled network of interconnected cells is formed that is subjected to the varying tensions developed by the irregular movements of the animal. In the horizontal plane the dorsal epithelium forms a layer of polygonal cells (Fig. 10 and 11), each bearing a single flagellum [6]. These cells partly overlap one another (Fig. 8) and are equipped with belt desmosomes (Fig. 11) as they generally exist in *Trichoplax* tissues. Many basal bodies and with them their proximal shafts are inclined towards the edge of the animal (Fig. 7). Relative to its surface area the layer of the slender ventral cells is more densely flagellated than the dorsal epithelium.

The moving flagella at the free edge of the animal can well be seen with phase contrast microscopy. They beat at 3–7 Hz as analyzed by high-speed cinematography with Nomarski interference optics (Fig. 3). The flagella are about 15 μm long. Metachronal beating, as defined by Machemer [14] and Sleigh [15], could not be identified. The power stroke is predominantly directed towards the middle region of the animal's dorsal surface to which food particles, for example unicellular algae or yeast cells, are transported by flagellar action. After addition of vanadate the flagellar beat slows down and completely ceases within several minutes. On the dorsal surface, one cannot discern the flagella while beating but they become clearly visible when considerably retarded or immobilized, than lying close to the organism's surface.

During the bright-field microscopy some inclusion bodies, particularly in the peripheral parts of the intact animal, are seen vibrating in a rhythm similar to the flagellar movement. These oscillations at several Hz and a few μm amplitude last from a fraction of a second to some seconds, are randomly directed and may be resumed after a short while; they often vary from one inclusion to another. Usually in a high-power field, there are 3–5 inclusions vibrating simultaneously. Thus, one has the impression of an irregular and alternating vibration or quivering of the coloured organelles. Relatively rare vibrations are seen on the dorsal surface remote from the edges, but in animals brought into the hypotonic medium (2.1% salt concentration) nearly all inclusions in the dorsal epithelium pulsate after twenty minutes. In Fig. 12 and 13, photographs from the video screen show displacements of some inclusion bodies. Occasionally a pulsating object gets out of the focal plane and disappears for seconds. If vanadate is

Fig. 7. Inclusion body near the dorsal surface. Flagellar basal body inclined towards the animal's edge situated on the right. Bar = 1 μm.

Fig. 8. Part of the dorsal ephithelium showing overlapping of flat cells. Bar = 1 μm.

Fig. 9. *Trichoplax* squash preparation, Giemsa-stained. Dark inclusion bodies of different size in about half of the cells. Bar = 10 μm.

Fig. 10. Horizontal section of the dorsal layer with polygonal cell circumferences. The flagellar cross-sections belong to flat cells underlying to a large extent the plane shown here, due to overlapping. Bar = 1 μm.

Fig. 11. Horizontal section of the dorsal epithelium with basal bodies belonging to the cells shown. Arrow head: Belt desmosome. Bar = 1 μm.

Fig. 12, a, b and 13a, b. Live *Trichoplax*. Photographs taken from the video screen showing five inclusion bodies (arrow heads) that, while vibrating, changed their positions relative to their neighbours within 0.1 sec. Bar = 10 μm.


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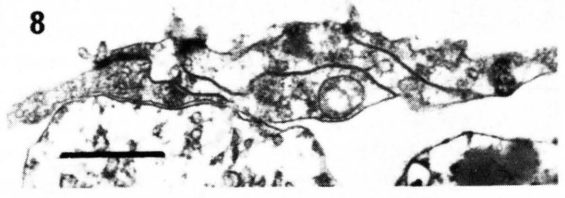
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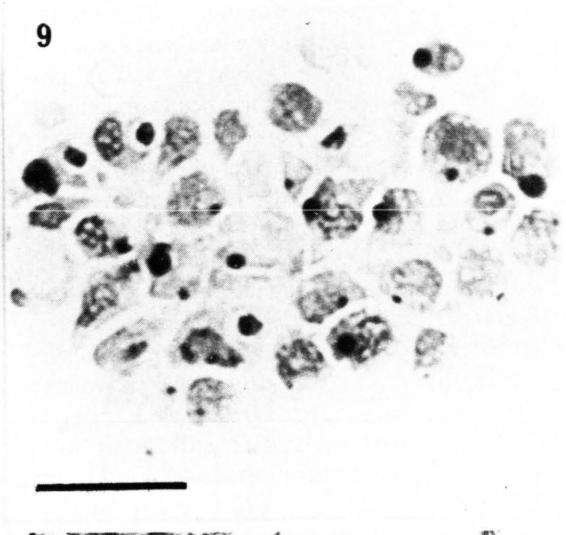
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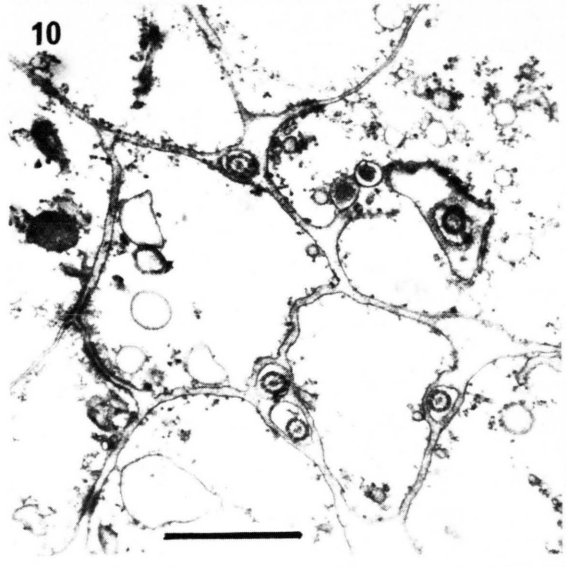
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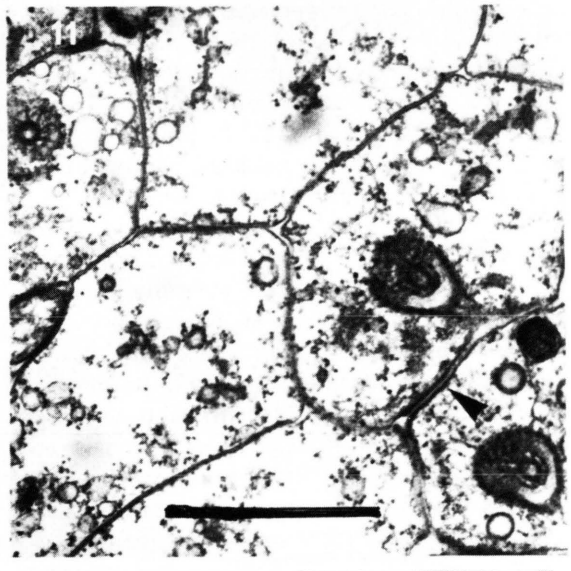

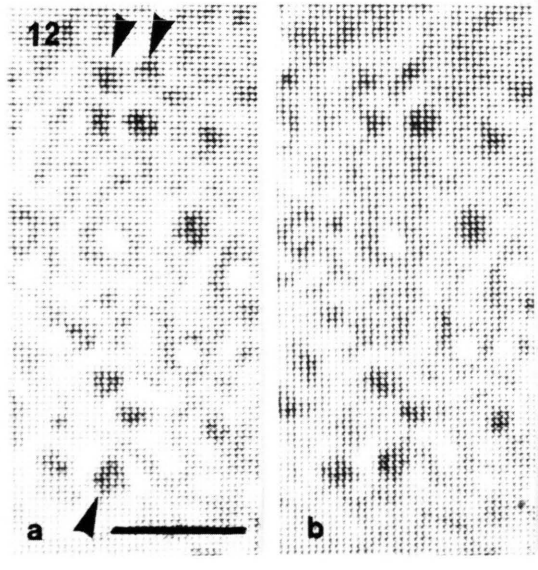
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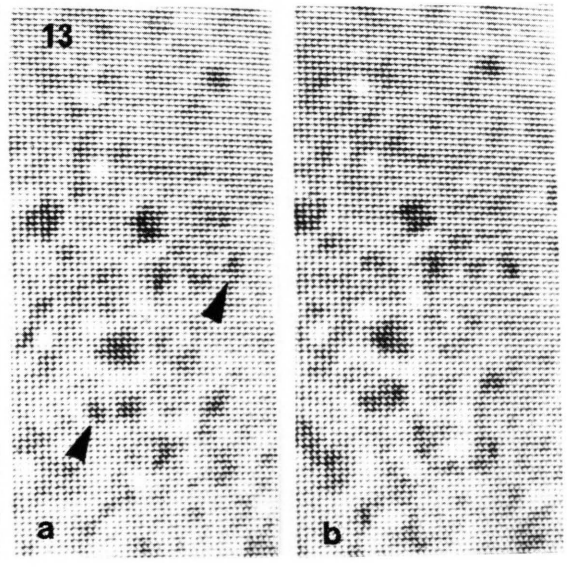
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administered as mentioned above, the beating of the flagella and the vibration of the inclusions cease altogether. – In the ventral epithelium similar motions of the inclusion bodies could not be seen with the inverted microscope.

Discussion

The inclusion bodies, or "concrement vacuoles" [3], probably contain waste materials from cell metabolism. As preliminary investigations suggest, the reddish colour of the inclusions is due to phycoerythrin, an auxiliary photosynthetic plant pigment of the bilin family, derived in *Trichoplax* from digested *Cryptomonas* algae (Wenderoth, in preparation). The inclusion bodies discussed here are of a characteristic ultrastructure and therefore cannot be mistaken for any other intracellular corpuscles, nor do they possess a motility of their own. That they are moved by flagellar force is the first impression one has while looking with high-power optics at the edge of the living animal: The inclusion bodies vibrate in the same rhythm as the flagella beat. This fact is proved by comparing the respective frequencies by high-speed cinematography and video recording, demonstrating undulating movements between 3 and 7 Hz for both objects.

Another argument in favor of a flagellar origin of the corpuscular motion is its slowing down and final standstill simultaneously with the progressive paralysis of the flagella by chemical means, for example by vanadate [16, 17]. This procedure also excludes Brownian motion from causing the intracellular vibrations and at the same time fulfils Rebhun's [18] claim that the selective destruction of the putative force-generating structure should halt the movements discussed. Metachronic beating as an expression of hydromechanical coupling cannot be expected in an arrangement of flagella whose basal bodies are several μm apart from one another. For the same reason, synchronous motions of the inclusions are improbable.

The basal body as a continuation of the flagellar shaft is anchored by subflagellar structures (fibres, microtubules, rootlets etc.) in the cell. Thus, the basal body functions as a fulcrum like the oarlock of a rowboat and transmits the counterforces developing with the power stroke to the cytoplasm where the basal body is embedded [19, 20]. In most examples of flagellated epithelia the single cell is sufficiently fixed by its neighbours so that considerable cytoplasmic deformations by flagellar forces are avoided. In the case of *Trichoplax* there is an extremely thin one-layer epithelium that rests largely upon the intercellular fluid which separates the different tissues. So structured, the dorsal epithelium forms a two-dimensional tensile sheet whose tensibility and elasticity are obvious as they allow transitory stretching of the whole animal to twice or thrice its former length. Although the basal body anchorage in the dorsal layer does not differ morphologically from the supporting elements described by Ruthmann *et al.* [7] in the ventral cells, this fixation is evidently not sufficient to withstand the counterforce which arises from flagellar beating. The claim of Pitelka [21] that the active motor organelle must remain firmly rooted in the cytoplasm is not applicable to *Trichoplax*. The flagellar power, fundamentally equal to that of a striated muscle fibre [22], is large enough to bring about the cytoplasmic displacement discussed here. Apparently it is not prevented by the presence of belt desmosomes (Fig. 11) whom Reed *et al.* [23] consider as sites of mechanical coupling to the neighbouring cells. That the inclusion bodies of the dorsal epithelium vibrate almost universally in animals exposed to a hypotonic medium is probably caused by the separation of the dorsal sheet from the connecting fibre cells; thus, the superficial elements have more freedom to follow the counterforces of the flagellar beat. Perpendicular sections through *Trichoplax* specimens kept in hypotonic solutions show a markedly expanded intercellular fluid space (Wenderoth, unpublished). Although the power stroke of the dorsal flagella is generally directed towards the centre of the animal's back the inclusions do not oscillate in a radial preference. In a deformable gel like the cytoplasm, a corpuscle next to the kinetoplast may be displaced in a direction different from that of the moving basal body. – Apparently the inclusions make their way with the same speed whether moved to or fro because movements at the cellular level proceed at low Reynold's numbers. Consequently inertia is negligible and viscoelasticity prevails. The cytoplasm accompanies the displacement of the basal body irrespective of its pulsation frequency, for hysteresis is small and compliance is great in soft tissues [24, 25]. The same physical

conditions support the elastic recoiling; the motion back may be enhanced by a weak inverse force during the recovery stroke [26].

That the inclusions oscillate only temporarily is possibly due to the ever-changing epithelial tension brought about by the varying contraction of the intermediate fibre cells. These elements are not only responsible for the continuous undirected movements of the whole animal but also contact locally the dorsal epithelium from below, reducing intermittently its motility. The same mechanism may work in the transient disappearance of the inclusions when the fibre cells pull part of the epithelium downward and out of the plane of view.

There are only a few examples, all of them in unicellular organisms, of dynamic cell changes connected to flagellar activities: Some algae vary their body shape simultaneously with the actual position of the flagella [27]; in his studies of algae and zoospores without cell walls, Melkonian [28] described a mechanism that prevents deformation of the cell by the action of the flagella: The position of the basal bodies changes with the phase of beating. In *Trichoplax* a similar design cannot be assumed. The basal bodies of the dorsal layer are frequently inclined towards the free edge of the animal (Fig. 7). It remains uncertain whether this inclined position is a constant arrangement connected with the direction of the power stroke – compare the implantation angles of the basal bodies in some flagellates – or just a temporary adaptation to the state of tissue tension [29].

Up to now it is not known whether the rhythmic and elastic cell deformation influences in any way cytoplasmic metabolism or structure. One could imagine mixing events in the cytogel as well as propagation of diffusion through the cell membrane. Even the expulsion of the "glossy corpuscles" [3] that are not shown in the figures presented here and that leave the *Trichoplax* body between the dorsal cells may be enhanced, since deformation of the cytoplasm will also affect the cell borders.

There is no suggestion that any other of the numerous intracellular motility phenomena discussed by Huitorel [30] and by Weiss *et al.* [31] pertain to the vibration of the inclusion bodies of *Trichoplax*. One can exclude cytoplasmic streaming and saltatory movements etc., either by their velocity, amplitude, or the failure of the corpuscles to return to their former sites. The high-frequency vibration described recently by Kamamyra and Kamiya [32] is restricted to the axoneme.

Nearly a century ago the discoverer of *Trichoplax*, F. E. Schulze [33], mentioned "a peculiar jerking ('zuckende') movement in the middle layer cells equipped with long filiform processes" [34]. Grell [2] confirmed this phenomenon but declared it an artifact produced by the pressure of the cover slip on the animal. However, Kuhl and Kuhl [35] in their painstaking investigations had been unable to verify the movement mentioned. It may be that Schulze really saw the vibration of the inclusion bodies but missed its true cause, the flagellar beat.

It seems possible that influences upon the surrounding cytoplasm due to flagellar movements occur also in other metazoan tissues, for example in the uniflagellated cells of veliger larvae, of *Actiniaria*, or in choanocytes of sponges. But it is *Trichoplax* that exhibits excellent conditions for such studies, since it bears transparent cells with coloured inclusion bodies whose vibrations, transmitted by the beating flagella, can easily be followed by conventional microscopy.

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