Table 1. Typing of paraproteins of SJL/J mice with antiserums specific for γ_1 and γ_2 mouse immunoglobulins.

Serum protein anomaly	Distribution of γ^1 and γ^2	
	Mice without overt disease	Diseased mice
Anomalous fast γG component	4 γ_1; 1 γ_2	7 γ_1
Monoclonal paraprotein + anomalous fast γ_1	1 γ_2	3 γ_2
Monoclonal (myeloma-like) paraprotein	3 γ_1; 2 γ_2	13 γ_1; 6 γ_2
Diclonal paraproteins	1 γ_1 and γ_2	5 γ_1 and γ_2

occasionally to a point where abnormality is no longer apparent or hypogammaglobulinemia supervenes.

Serums from 46 SJL/J ♀♀ with discrete serum protein anomalies were typed with antiserums specific for γ_1, γ_2, γA and γM (provided by J. L. Fahey) (*9*). The results are summarized in Table 1, and examples are illustrated in Fig. 2. With one exception, the anomalous, fast-migrating γG typed as γ_1. There were 34 mice with myeloma-like paraproteins; 16 of these were γ_1 and 12 were γ_2; the other six mice each had two paraproteins, γ_1 and γ_2. No case showed two myeloma-like paraproteins of the *same* antigenic type. The fast-migrating γG component was associated with a separate γ_2 monoclonal increase in four cases but was not found associated with a separate γ_1 monoclonal increase; this supports the view that the fast γ component is an earlier stage of a γ_1 monoclonal increase. There was no instance of a myeloma-like increase in γA or γM (in contrast to the frequency of γA paraproteins produced by myelomas of the mouse). As a rule, mice with the isolated fast γG change showed an elevation in γA, whereas serums with a monoclonal increase of γ_1 or γ_2 showed a decrease in γA and γM. Urines from four mice with the anomalous fast γG and four mice with monoclonal increases were concentrated and tested for paraproteins. Only one showed an anomalous urinary protein; this was a fast-migrating γ_1 similar to that in the serum. Paraproteins are not found in mice bearing transplanted lines. However, these animals may develop marked hypogammaglobulinemia (Fig. 2). (No serum protein abnormalities were observed in 123 normal SJL/J mice aged 6 to 12 weeks.)

The lesions of this disease, with admixture of neoplastic and apparently reactive cells, prompts comparison with Hodgkin's disease, although the striking changes in serum proteins are not seen in Hodgkin's disease. With regard to etiology of the disease (*10*) we may suggest three general possibilities (which do not exclude initial causation by a virus), as follows.

First, it might be a polyclonal malignancy affecting various types of cells; the invariable emergence of only the reticulum cell in transplanted lines is against this interpretation.

Second, it might be a monoclonal malignancy of reticulum cells with an exuberant host reaction to the malignant cells or their products, which would explain the frequent lack of transplantability. However, transplanted lines do not elicit a proliferative response of plasma cells such as is seen in primary cases, and the serum protein abnormalities that are seen in primary cases take the form of discrete paraproteins suggestive of abnormal cellular proliferation rather than diffuse increases indicative of reactive hyperplasia.

Third, the disease might be basically a proliferative disorder of certain clones of reticulum cells that at an early stage have the capacity to differentiate into plasma cells; as the disease progresses their capacity to differentiate is lost, mature plasma cells and the accompanying paraproteinemia disappear, and finally, in many cases, an autonomous transplantable reticulum cell sarcoma is established. This is the possibility that we believe best fits our present knowledge of the disease.

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Substructure of Certain Cytoplasmic Microtubules: An Electron Microscopic Study

Abstract. *Negatively stained cytoplasmic microtubules of lung-fluke sperm show a helical structure that is not found in peripheral doublet tubules of axial units. In transverse sections, the wall of such microtubules appears to comprise about eight subunits.*

The structure, relationships to other cell organelles, and possible functions of cytoplasmic microtubules have received much attention since Slautterback's (*1*) discussion of their occurrence and relationships in hydra. My work deals with the substructure of microtubular elements in a spermatozoon as seen in both sectioned and negatively stained material.

Trematode worms (*Haematoloechus medioplexus*) were obtained from the lungs of *Rana pipiens*. Pieces of the body, containing the seminal receptacle or testes, were fixed in cold 6 percent glutaraldehyde (*2*) buffered with *s*-collidine (*3*) at *p*H 7.4 to 7.7. Material was embedded in Epon 812 (*4*); sections were cut with glass or diamond knives and stained with lead citrate (*5*), uranyl acetate, or both. Negative-staining was achieved with 1 percent sodium phosphotungstate (*6*). Sperm were obtained from seminal receptacles or testes teased in a drop of sodium phosphotungstate. Specimen screens, coated with Parlodion and a thin film of amorphous carbon, were then flooded with this fluid by means of a micropipette. Seminal receptacles or testes to be ultrasonically disrupted were collected in about 1 ml of sodium phosphotungstate and treated for 1 to 4 minutes with a Bronwill Biosonik generator at 75 percent maximum probe intensity (125 watts). Micrographs were made

with an RCA EMU-3H electron microscope; for purposes of measurement, the microscope was calibrated with two different carbon grating replicas immediately after a series of exposures.

Lung-fluke sperm are less than a micron in diameter and are about 400 microns long. Two sets of cytoplasmic microtubules lie beneath the plasma membrane and run the length of the cell (Fig. 1); paired axial units are present but lack typical central elements. The microtubules are 225 to 250 Å in diameter. In longitudinal sections their margins appear saw-toothed or crinkled, and often the microtubules appear crossbanded (Fig. 2). The crossbands are usually slightly oblique, and careful examination of their relation to one another suggests that the microtubules have a helical structure. As seen in favorable transverse sections, the wall of the microtubule seems to be made up of eight, or so, subunits about 65 Å in diameter (Figs. 3 and 4). Although accurate counting of such subunits is difficult, rotation photographs which enhance radial periodicity (*7*) show strong reinforcement when printed by rotation through successive angular increments of $360°/n$ when n equals 8 (Fig. 5, a–c).

Micrographs of negatively stained microtubules of the sperm suggest that they resist bending, for flexed microtubules are not frequently seen (Fig. 6). The straight pieces of broken microtubules are commonly seen, with the breaks almost always having occurred in a transverse direction. No microtubules split longitudinally were observed. At high magnification negatively stained microtubules appear as tightly coiled springs, with the gyres

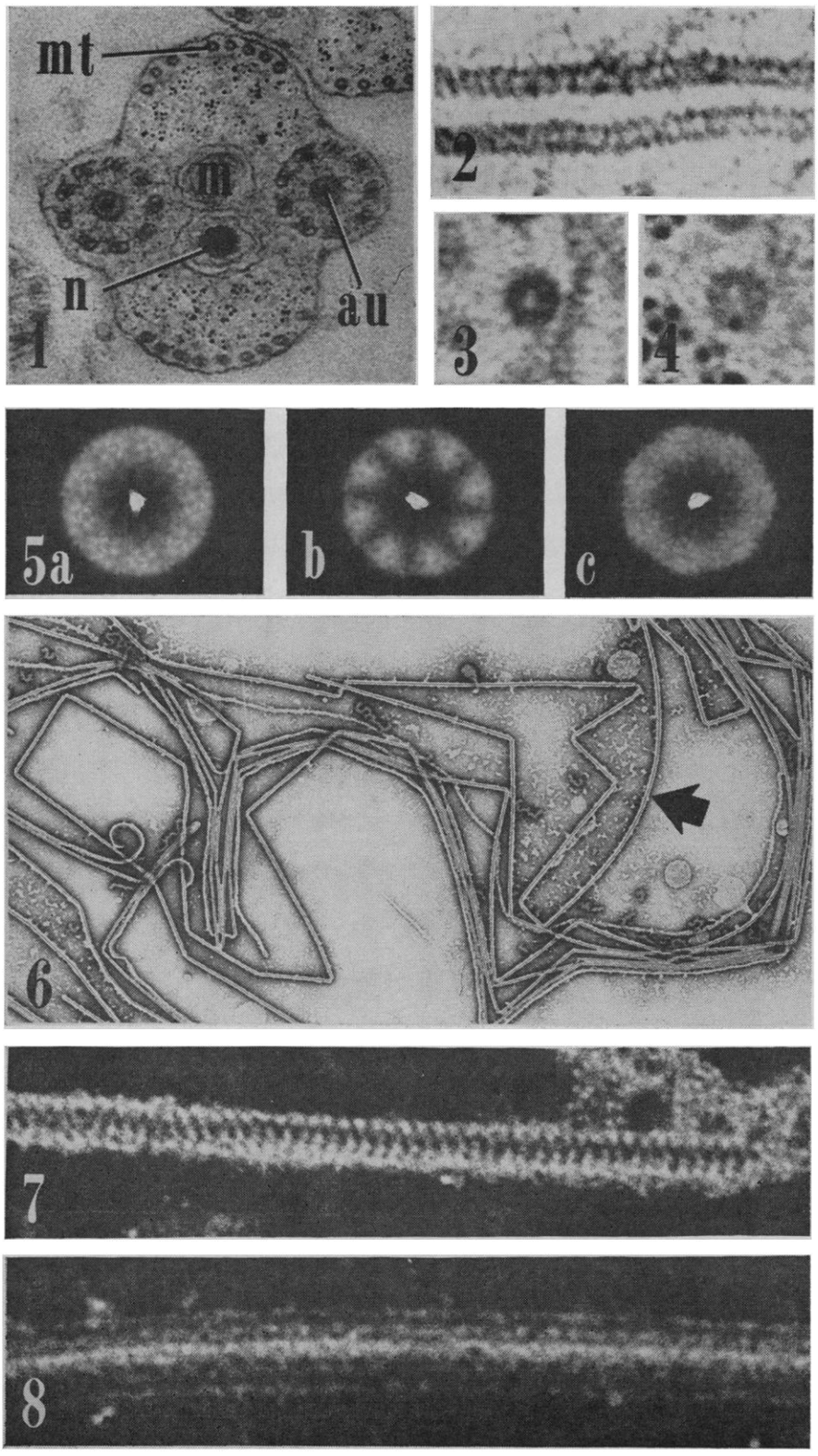

Fig. 1. Transverse section of lung-fluke sperm through head region. *n*, Nuclear material; *m*, mitochondrion; *au*, paired axial units; and *mt*, two sets of microtubules, one upper and one lower, just beneath plasma membrane (× 80,000). Fig. 2. Longitudinal section of cytoplasmic microtubules seen in sectioned material. Note crossbands and irregular profiles (× 237,600). Figs. 3 and 4. Microtubules seen in transverse section. Note subunits; dense granule in wall of microtubule in Fig. 4 is an artifact (× 332,600). Fig. 5. Multiple-exposure rotation photographs of transverse section of microtubule shown in Fig. 4, where (a) n equals 9, (b) n equals 8, and (c) n equals 7 (n equals the number of arcs in a circle). Greatest optical reinforcement is in (b), suggesting the presence of eight subunits. Fig. 6. Negatively stained microtubules from ruptured sperm. Arrow indicates flexed microtubule, which is not often seen. Note sharp angles formed by broken microtubules. Curled elements at center-left are pieces of plasma membrane and cortex of sperm (× 25,900). Fig. 7. Micrograph of microtubule in negatively stained preparation at high magnification. Note obvious helical structure. Broken end of microtubule is at right, and particles in vicinity of break are about 50 Å in diameter (× 289,800). Fig. 8. Micrograph of negatively stained doublet tubule of axial unit at high magnification. Note longitudinally oriented elements and dissimilarity from microtubules shown in Fig. 7 (× 289,800).

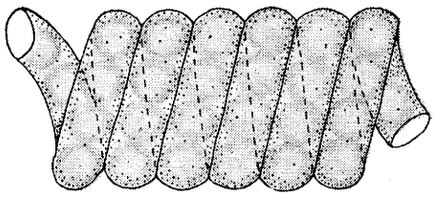

Fig. 9. Diagrammatic representation of cytoplasmic microtubule of lung-fluke sperm showing proposed structure. Pitch of the helix is about 15 degrees.

repeating at intervals of about 70 Å (Fig. 7). The pitch of the helix varies from 10 to 25 degrees. Sodium phosphotungstate does not appear to fill the lumen of the microtubule, even when broken so that "open" ends are exposed, and the appearance of the helical pattern is apparently due to the surface deposition of sodium phosphotungstate in the grooves between contiguous gyres. Thus, the helical structure suggested by longitudinal sections of microtubules is confirmed by negatively-stained preparations, even though negatively-stained microtubules show little evidence of the 65-Å subunits seen in transverse sections. In many broken microtubules, however, there appears to have been a disorientation of the helical structure at the broken ends. The result is a mass of particulate material at the free end, the mass being continuous with the microtubule proper (Fig. 7). The particles forming the mass appear to be roughly spherical, and they have a diameter of about 50 Å. It is probable that they represent the subunits seen in transverse sections of microtubules.

Axial units were easily recognized in negatively stained preparations. The nine peripheral doublet tubules of an axial unit do not show helical structure and, indeed, are quite different in appearance from the cytoplasmic microtubules (Fig. 8). They measure about 450 Å in width, and, unlike the microtubules, their lumina usually appear to be filled with sodium phosphotungstate. Negatively stained doublet tubules of sperm tails reportedly (*8*) have walls composed of 10 or 11 longitudinally oriented filaments, which are regularly beaded. Such well-defined filaments were not seen in doublets of axial units of lung-fluke sperm, although longitudinally oriented elements appear to be present. In Fig. 8, several such elements can be seen; the lumina of the two tubules are opaque, being filled with sodium phosphotungstate, and the longitudinal conection between the tubules is electron-lucent.

Cytoplasmic microtubules of lung-fluke sperm appear to be quite structurally stable, for with increased periods of sonic disruption they are broken into shorter and shorter fragments, which retain their basic helical structure after most other cell organelles have disintegrated. For example, after 4 minutes of treatment under the conditions designated, only short pieces of microtubules and pieces of the unique central core of axial units were recognized (doublet tubules of axial units were not seen).

Subunits forming the wall of these microtubules appear to be larger in diameter and fewer in number than those described for other such microtubular elements (*8, 9*). Assuming the subunits are globular, one can imagine them arranged in a helical pattern (Fig. 9), and I suggest that the microtubules I have described are so structured. Sandborn *et al.* (*10*) suggest that microtubules and microfilaments may be derived from one another, with globular units measuring 50 to 60 Å serving as building-blocks. While the helical structure of a lung-fluke sperm microtubule may result from the coiling and cross-bonding of a single microfilament, it appears that such is not the case for doublet tubules of axial units.

At least one function of the cytoplasmic microtubules of lung-fluke sperm is support, for living sperm show restricted flexing movements and give the appearance of being somewhat rigid or stiff. These qualities could also be used to describe their cytoplasmic microtubules.

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Sweet-Sensitive Protein from Bovine Taste Buds: Isolation and Assay

Abstract. *Using refractometry and ultraviolet-difference spectroscopy to indicate interaction between proteins and compounds of low molecular weight, we found a protein fraction in bovine tongue extracts that complexes sugars and saccharin. The strengths of the complexes parallel the degrees of sweetness of the compounds, and the effects of* p*H upon formation of complexes parallel the effects of* p*H upon sensitivity of taste buds to sweet compounds in vivo.*

In higher animals, a chemoceptor system is responsible for the sense of taste (gustation). While electrophysiological studies have firmly established the localization on the tongue of taste buds responding to sweet, sour, salty, and bitter substances, our understanding of the biochemistry of taste is rudimentary. Attempts to correlate the tastes of various compounds with their chemical structures or physical properties have met with only limited success (*1*). The nature of the initial interaction between taste stimuli and taste receptor has been the subject of considerable speculation (*2*). Attempts to correlate taste sensitivity with enzyme activities have been unsuccessful, and there are rather convincing arguments against taste being primarily enzymic in nature (*2, 3*). Indeed, available evidence strongly suggests that the initial interaction is formation of a weak complex between the stimulus compound and some receptor molecule at or near the surface of the taste bud (*2*).

Thus we tried to extract and purify "receptor molecules" from taste buds. In principle, the problems should be exactly analogous to those involved in extraction and purification of an enzyme, except that with enzymes one must seek fractions catalyzing a given reaction, while we had to seek fractions forming complexes with compounds of a given taste. Following the