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USEFULNESS OF TETRAMETHYLSILANE IN THE PREPARATION OF HELMINTH PARASITES FOR SCANNING ELECTRON MICROSCOPY

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Helminth parasites belonging to different groups and from different hosts were prepared for scanning electron microscopy following a simple air-drying technique using an organo silicon compound, tetramethylsilane. The results when compared with critical-point drying were found to be similar, while the control specimens prepared by simple air drying showed distortion, wrinkling and shrinkage in different parts of the body. The method, which is economical and has already been tried successfully for trematodes, may be used as an alternative technique to critical point drying for most helminths. However, the success of the method will depend on the proper fixation schedule, since the chemical nature of the body surface of helminths belonging to different groups would be different.

INTRODUCTION

A wide range of preparative techniques has been utilized to prepare the vast array of specimens for scanning electron microscopy. It has been suggested that no single scheme is applicable to all specimens; rather each specimen may require different procedures to best preserve the surface and/or subsurface morphology.

The usual procedure for preparation of biological specimens for SEM is fixing in 2.5-3% glutaraldehyde in 0.1 M sodium cacodylate buffer, post fixation in 1% buffered Osmiun tetroxide, dehydration through concentrations of acetone, and drying through critical point drier. However, for helminths belonging to trematode, nematode and cestode groups, very different fixation schedules were employed. While Tulloch *et al.* (1977) used a simple 10% formalin fixative, Senft and Gibler (1977) employed sequential glutaraldehyde and osmium fixatives followed by repetitive thiocarbohydrazide (TCH) and Osmium tetroxide treatments for trematodes. Nicholls *et al.* (1985) used 10% phosphate buffered formalin followed by 1% Osmium tetroxide in 0.13 M Milloning's sodium phosphate/NaOH buffer containing 0.06 M glucose for nematodes. Yamane e*t al.* (1982) used glutaraldehyde followed by Osmium tetroxide for cestodes. Specimens in all the cases were dried by the critical-point drying (CPD) technique.

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CPD and freeze drying techniques are the standard techniques for drying most of the biological specimens (Anderson 1951, Boyde and Wood 1969, Cohen 1974, Hayat 1978, Echin and Moreton 1973, Nei 1974, Osatake *et al.* 1980, Inoue and Osatake 1988), since most of them, except for a few hard structures cannot be prepared by simple air-drying as it causes shrinkage, wrinkling and distortion of the tissue, rendering it unsuitable for SEM studies.

Among the helminths, nematodes which posses a rigid and highly resistant cuticle can be studied by air-drying. Prior to air-drying, the specimens are fixed in formaldehyde, followed by dehydration through increasing concentration of acetone and subsequent glycerine or resin replacement (Allison *et al.* 1972, Maugel *et al.* 1980). For trematodes and cestodes which have softer body surface, CPD and freeze-drying remain the only means for drying them. However, both these techniques suffer from some drawbacks; while the CPD causes considerable thermal and pressure stresses in tissue, takes quite a long time and may also promote extraction of additional cellular components in the transitional fluid (Gunning and Crang 1984), the main disadvantages of freeze drying techniques are the surface damage caused by ice crystal formation and relatively long time taken to dry the tissue (Boyde 1978).

In an attempt to find alternative drying technique, a simpler method was reported, which gives results comparable to CPD (Dey et al. 1989) in a trematode. However, as helminth parasites belonging to different groups possess different composition of integument or different degree of softness, the applicability of this method to different groups of helminths is highly desired.

The present investigation gives a detailed study on the application of this method to trematodes, cestodes and nematodes. The effectual of different fixatives with the technique has also been investigated.

MATERIALS AND METHODS

After washing in 0.85% normal saline, one set each of the specimens was fixed in 2%, 4% and 10% cold, neutral phosphate buffered formalin for 12 hr. Another set was fixed in 3% glutaraldehyde in 0.1M Sodium cacodylate buffer for 6 hr at 4° C. Following the primary fixation, the specimens were washed in the respective buffers and post fixed in 1% buffered Osmium tetroxide for 1 hr. Dehydration was carried out using ascending concentrations of acetone. After the final treatment of the specimens in dry acetone, one set was quickly transferred to a vial containing Tetramethylsilane [$(CH_3)_4Si$, TMS, boiling point 26,3° C, surface tension: 10.3 dynes/cm at 20° C]. The material was dipped in TMS for 10 minutes and then allowed to dry at room temperature (25° C). One set used as control was allowed to dry directly from dry acetone instead of TMS. Specimens for CPD were prepared using acetone as the intermediate fluid and CO_2 as the transitional fluid. The dry samples were coated with gold in Fine coat Ion sputter JFC-1100 (JEOL) and observed with the scanning electron microscope JSM-35CF (JEOL) with an accelerating voltage of 10-15 Kv.

RESULTS

The specimens dried from TMS in all the different fixation schemes showed better results than the corresponding control samples, dried directly from acetone. Excellent surface details were preserved in TMS treated samples and were similar to CPD preparation, whereas those prepared by simple air drying showed distortion, wrinkling and shrinkage of some surface structures.

Among all the fixatives used the best result was observed in 4% formaldehyde followed by post fixation in 1% Osmium tetroxide. The specimens prepared with other fixations such as 2% and 10% formalin revealed slight destortion of structures. However, the advantage of TMS treatment over that of simple air-drying was prominent in all samples irrespective of the fixative used.

Trematode

The observations in respect of both flukes, viz. *Artyfechinostomum* and *Schistosoma* reveal that the TMS treated (Figs. 1, 2, 7 & 8) and CPD (Figs. 3, 4, 9 & 10) specimens showed almost similar extent of clarity of fine structures, while air drying (Figs. 5, 6, 11 & 12) caused much distortion and shrinkage rendering the specimens unsuitable for SEM study.

Cestode

The scolex (Figs. 13, 15) and proglottids (Figs. 14, 16) of *Raillietina* sp., prepared by both TMS and CPD showed their undistorted intact structure, whereas a distinct shrinkage was observed in these structures following air-dried preparation (Figs. 17, 18).

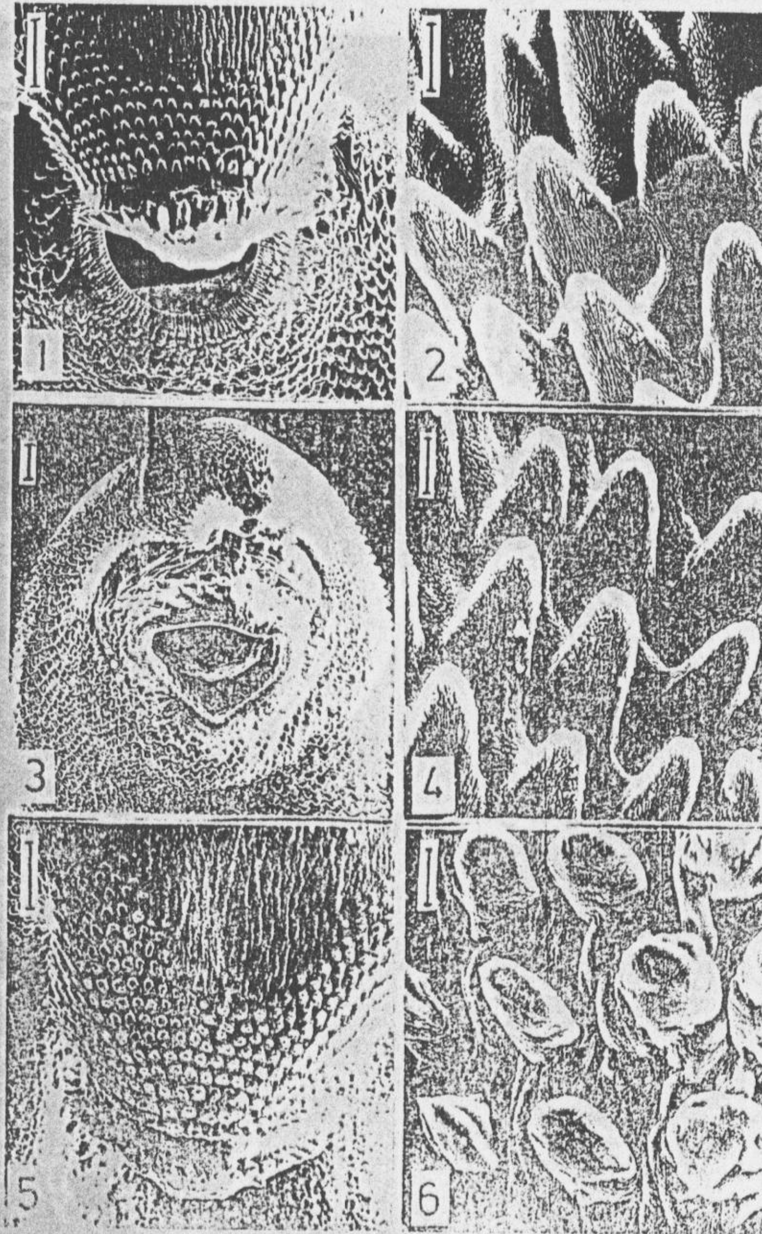
Nematode

Heterakis sp. prepared by TMS (Figs. 19, 20) and CPD (Figs. 21, 22) showed similar clarity of mouth parts as well as the general body surface, whereas the air-dried specimen (Figs. 23, 24) showed total distortion of the whole body.

DISCUSSION

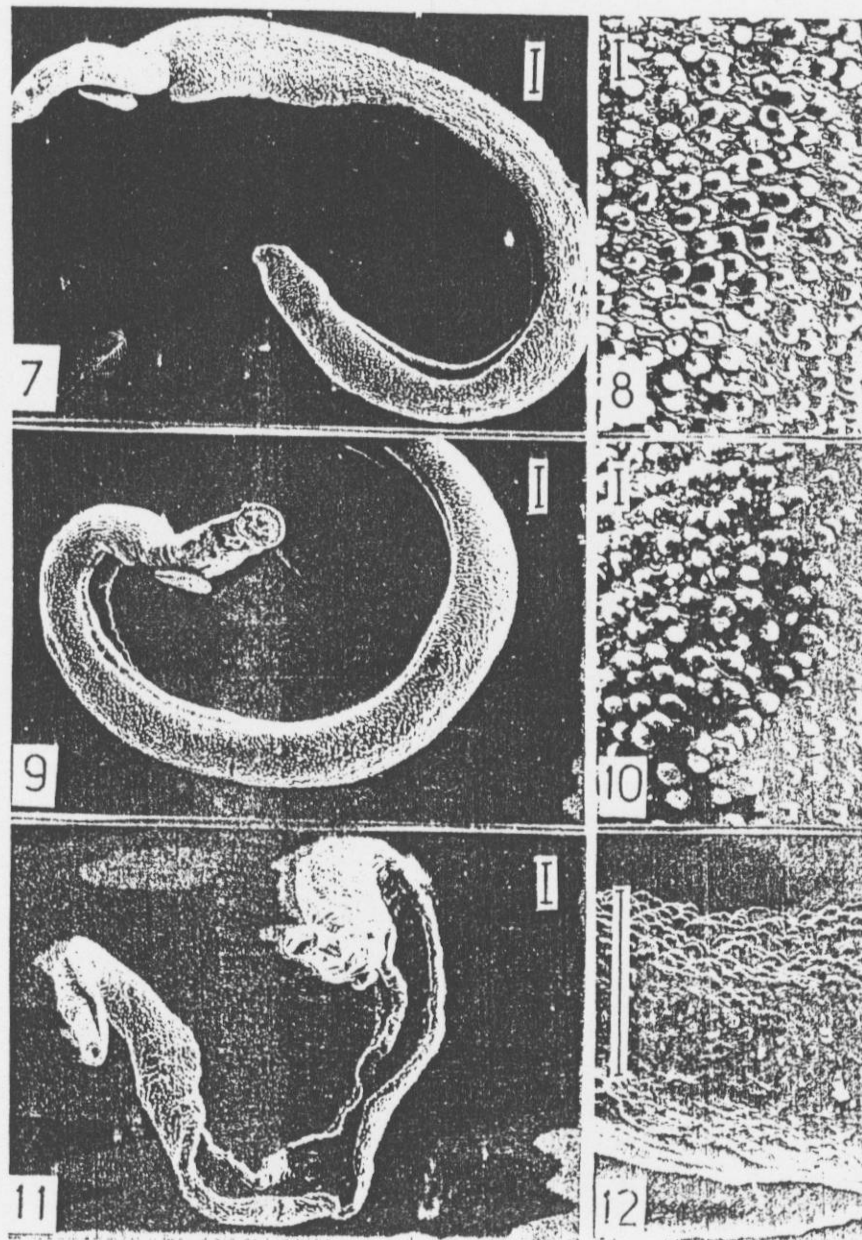
Scanning electron microscopy plays a very important role in taxonomy and morphological studies of helminthes, because of its large depth of field and high resolving power. The most critical part of SEM is the preparation of the specimen. Adequate fixation, proper dehydration and uniform metal coating are important in determining the quality of a micrograph.

The characteristic dome shaped papillae or bosses of *Schistosoma* spp. (Hick and Newman 1977, Probert and Awad 1987), spines in the echinostomid fluke (Fried and Fujino 1984), contour of proglottid and suckers of the cestode (Yamane *et al.* 1982) and oral lips and denticles of nematodes (Gibbons 1986) were retained only in TMS treated specimens and critical-point–dried preparations.



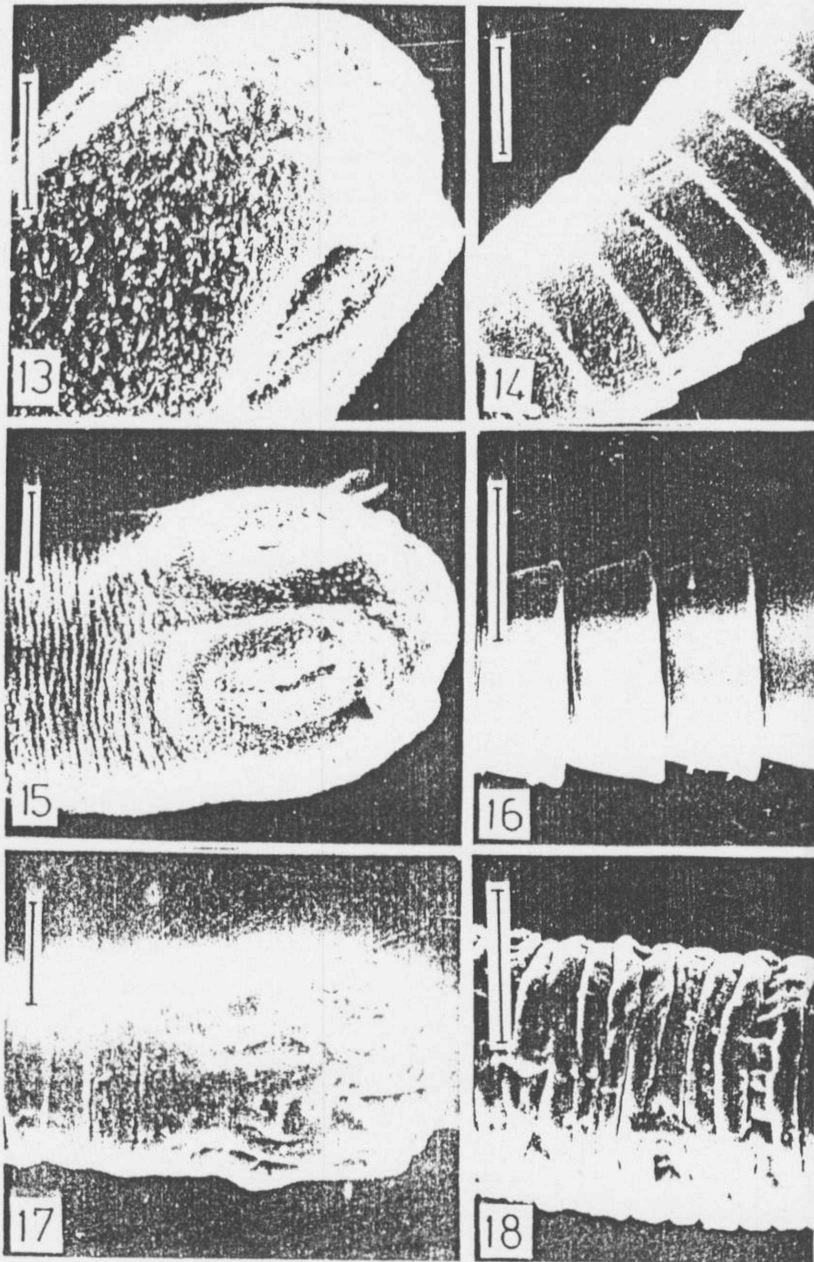
Figs. 1-6 Scanning electron micrographs of *Artyfechinostomum* Sp.

Fig. 1 Anterior region of worm showing collar spines and body spination (TMS treated) Scale bar = 100 µm). Fig. 2. Enlarged view of body spines (TMS treated) (Scale bar = 10 µm). Fig. 3. Anterior region of worm (CPD preparation) (Scale bar = 100 µm). Fig. 4. Enlarged view of body spines (CPD preparation) (Scale bar = 10 µm). Fig. 5. Anterior portion of specimen (air dried) (Scale bar = 100 µm). Fig. 6. Body spines in an enlarged view (air dried) (Scale bar = 10 µm).



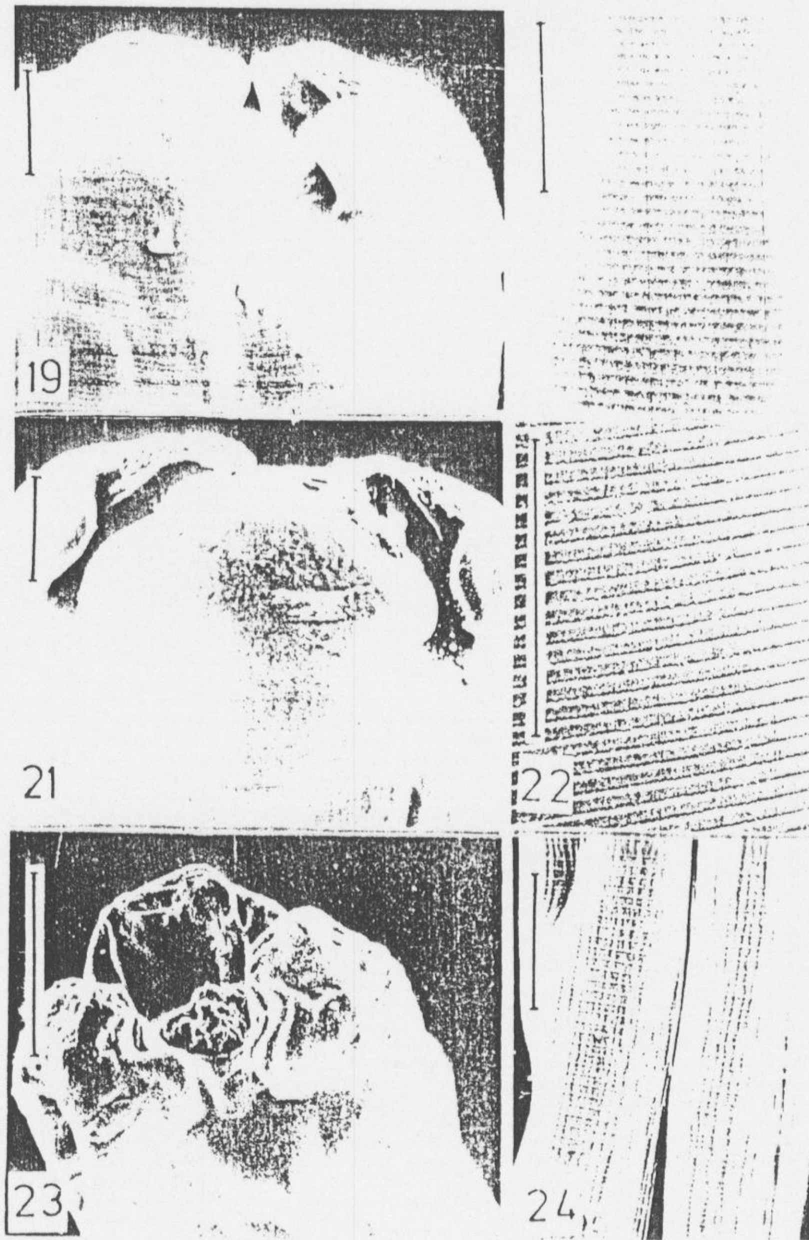
Figs. 7-12 Scanning electron micrographs of *Schistosoma* sp.

Fig. 7. Whole worm (TMS treated) (Scale bar = 100 µm). Fig. 8. Enlarged view of dorsal tegumental papillae (TMS treated) (Scale bar = 10 µm). Fig. 9. Whole worm (CPD preparation) (Scale bar = 100 µm). Fig. 10. Enlarged view of tegumental papillae (CPD preparation) (Scale bar = 10 µm). Fig. 11. Whole worm (air dried) (Scale bar = 100 µm) 12. Body surface (Air dried) (Scale bar = 100 µm)



Figs. 13-18 Scanning electron micrographs of *Raillietina* sp.

Fig. 13. Scolex of worm (TMS treated) (Scale bar = 100 µm). Fig. 14. Proglottids (TMS treated) (Scale bar = 100 µm). Fig. 15. Scolex (CPD preparation) (Scale bar = 40 µm). Fig. 16. Proglottids (CPD preparation) (Scale bar = 40 µm). Fig. 17. Scolex with few proglottids (air dried) (Scale bar = 40 µm). Fig. 18. Proglotids (ait dried) (Scale bar = 40 µm).



Figs. 19-24 Scanning electron micrographs of *Heterakis* sp.

Fig. 19. Mouth part of worm showing lips and denticles (TMS treated) (Scale bar = 40 μm). Fig. 20. Body surface in an enlarged view (TMS treated) (Scale bar = 100 μm). Fig. 21. Mouth part (CPD preparation) (Scale bar = 40 μm). Fig. 22. Enlarged view of body surface (CPD preparation) (Scale bar = 100 μm). Fig. 23. Mouth part (air dried) (Scale bar = 100 μm). Fig. 24. Body surface (air dried) (Scale bar = 100 μm).

In simple air drying, the tissue shows severe shrinkage and distortion because of the disruptive effect of the surface tension of acetone from which they are dried. Slow rate of evaporation during the process is also responsible for the unfavourable effects. These points were taken into consideration while studying the ideal situation in which the tissue could be dried without severe shrinkage and distortion. It was found that TMS, because of its low boiling point and being chemically inert, seems an ideal fluid for the purpose.

The significance of the method is that it is simple and economical and it avoids delay involved in drying by conventional critical-point an freez-drying techniques.

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