PAP prevents the replication of some animal viruses<sup>22,23</sup>, and therefore it is possible that the inhibitors described here have similar action.

These inhibitors are probably one type of compound amongst many substances controlling virus replication. Virus inhibitors separated from plants have been variously identified as proteins, glycoproteins and polysaccharides<sup>2</sup>. Extracts with lectin-like properties have also been implicated in antiviral activity<sup>4</sup> and, interestingly, some lectins also inhibit protein synthesis11. Furthermore, evidence is accumulating that some antiviral compounds are induced in plants in response to virus infection<sup>24,25</sup>; such compounds, like the antiviral compound from Dianthus sp. 26, have been compared to interferon<sup>28</sup>.

The antiviral activity of plant extracts is exerted largely against viruses in plants different from the ones from which the extracts are prepared and this has led to the conclusion that they act on the host plant rather than on the virus<sup>29</sup>. If these antiviral proteins act enzymically on ribosomes<sup>9</sup>, they should act on ribosomes different to their own; this has been shown with PAP which inactivates ribosomes from wheat germ and from cowpea but not those pokeweed8. It may be concluded that many, and possibly all, plants contain enzymic proteins either free (like PAP), or similar to the A chain protein of ricin and related toxins, being bound to a 2nd B chain protein. Such enzymic proteins, capable of recognizing and inactivating foreign ribosomes, may be a selective RNAse of the type recently demonstrated in eukaryotic cells (Hela cells)<sup>30</sup>.

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## Observations on calcareous corpuscles using a scanning electron microscope

A. I. Ishii, K. Morimoto and M. Sano

Department of Parasitology, Hamamatsu University School of Medicine, Hamamatsu (Japan), 2 May 1980

Summary. Calcareous corpuscles were observed in a scanning electron microscope, and the presence of Ca was examined by means of elemental X-ray analysis.

Calcareous bodies, round or oval in shape, often described as 'calcareous corpuscles' in the literature, are known to occur in the parenchyma of cestodes and to store large amounts of calcium and magnesium carbonates in addition to phosphates and phospholipids<sup>1,2</sup>. It is reported that the binding of the Ca<sup>2+</sup> to phospholipids is stronger than that of Mg<sup>2+3</sup>. Several workers have attempted to study: a) the formation of these structures in different species of cestodes by the use of the light and electron microscope<sup>4-10</sup>, and b) their inorganic composition using X-ray diffraction or emission spectrographic methods<sup>11-14</sup>. In this study the shape of calcareous corpuscles was examined using the scanning electron microscope (SEM), and the presence of Ca was investigated by means of elemental X-ray analysis. Materials and methods. Plerocercoid of Diphyllobothrium erinacei obtained from the snake, Rhabdophis tigrinus, was employed as the material. Plerocercoid was fixed with formalin solution and cut with a razor to small pieces. After washing with distilled water to remove formalin, the specimens were freeze-dried and coated with carbon. The specimens thus prepared were examined in an ISI-30 scanning electron microscope, and elemental X-ray analysis was performed<sup>15</sup>.

Result and discussion. There are several studies regarding the structure of the calcareous corpuscles using the light microscope, and the transmission electron microscope (TEM). There are, however, few reports on investigations using scanning electron microscopy. A scanning electron micrograph of the parenchyma containing the corpuscles is shown in figure 1. At high magnification of the area outlined in figure 1, calcareous corpuscles can be clearly observed as spherical or ellipsoidal bodies in the parenchyma (figure 2). Their surfaces appear to be smooth. It is reported that their diameter may be up to 30  $\mu m^{6,16}$ . Using the SEM they were also seen to vary in size, the range was between 10 and 20 µm. The chemical composition of calcareous corpuscles has been examined with histochemical methods<sup>7,17</sup>, X-ray diffraction and emission spectrogra-

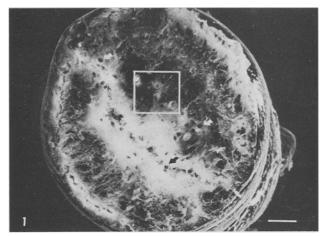


Fig. 1. Scanning electron micrograph of parenchyma of plerocercoid of D. erinacei showing small corpuscles (arrows). Bar: 100 µm.

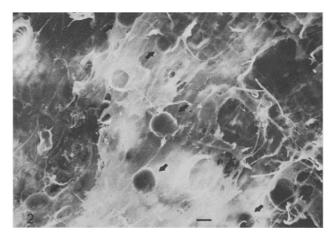
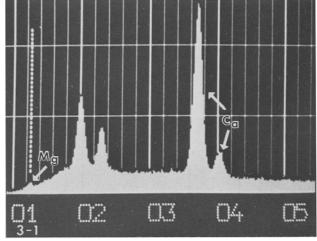


Fig. 2. Scanning electron micrograph at high magnification of parenchyma outlined in the box in figure 1. Here corpuscles can be clearly observed as spherical or ellipsoidal materials (arrows). Bar: 10 μm.

phy. In this study calcium and magnesium, the major cations in the corpuscles, were examined by means of elemental X-ray analysis. The X-ray spectrum is shown in figure 3-1. The presence of Ca was clearly demonstrated by sharp peaks, although the peak of Mg was weak. The X-ray image (figure 3-2) of  $Ca-K_a$  emission from the same area in figure 2 coincided with the sites of corpuscles. But in the case of Mg, the X-ray image could not be distinguished from the X-ray background signals. Although it is unclear why the peak of Mg was weak on the X-ray spectrum, it may be because the sample preparation process was not adequate for this elemental X-ray analysis or that there was originally only a very small amount of Mg in the calcareous corpuscles of this specimen. In order to minimize the movements of components cryo-SEM<sup>18</sup> would be a better technique for such studies, and it is also necessary to apply elemental X-ray analysis and cryomicrotomy19 to obtain detailed information on calcareous corpuscle formation by the use of TEM.

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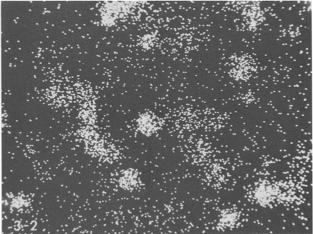


Fig. 3. 3-1 X-ray spectrum of calcareous corpuscle showing the sharp peaks of Ca and the weak peak of Mg. 3-2 X-ray image of Ca- $K_a$  emission from the area shown in figure 2.

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