part resembles type 6, is the only type in the region that extends to places receiving less than 1250 mm average annual rainfall[9]. After extensive search, type 6 is detected only from the following habitat: Telefomin district, Papua New Guinea; 5° 10′ S, 141° 35′ E, altitude about 1700 m; rainfall in excess of 2500 mm average annual; vegetation, mid-montane rainforest dominated by *Nothofagus* spp.; source of hyphae, different sorts of undetermined meso- and macrophylls from the forest floor leaf litter. A microscope slide of the illustrated specimen (Telefomin No. 394) is available from the Botany Department, Adelaide University, South Australia.

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Electron probe microanalysis of pyroantimonate precipitates in the cilia of *Paramecium*

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Summary. Electron-dense deposits were observed at the bases of the cilia of *Paramecium* fixed in 1% OsO_4 solution containing 2% potassium pyroantimonate. The deposits were shown to contain Ca and Sb by X-ray microanalyzer.

Ca ions are now known to modify ciliary and flagellar movement both in protozoa and in metazoa, e.g. the ciliary reversal response in ciliates and the ciliary arrest response in the lamellibranch gill. *Paramecium* extracted with Triton X-100 and reactivated with Mg-ATP swam backwards when the Ca ion concentration was raised above 10^{-6} M[1]. Similarly, extracted lateral cilia of *Mytilus* gill showed an arrest response that was controlled by Ca ions[2,3]. In the living cell, such an increase in Ca ion concentration could result directly from the influx of Ca ions across the excited cell membrane[4] and/or Ca ions necessary for the ciliary response could be liberated from some intracellular binding sites[5]. Recent electron microscopic studies on *Paramecium* fixed in glutaraldehyde solution containing $CaCl_2$ (glutaraldehyde method) have shown the localization of electron dense deposits at the bases of the cilia[6,7] and it was clearly demonstrated that the deposits consisted of Ca and P[8]. In the above-mentioned experiment, however, the fixative contained considerable amounts of Ca ions, which were supposed to bind with the sites at the base of the cilia.

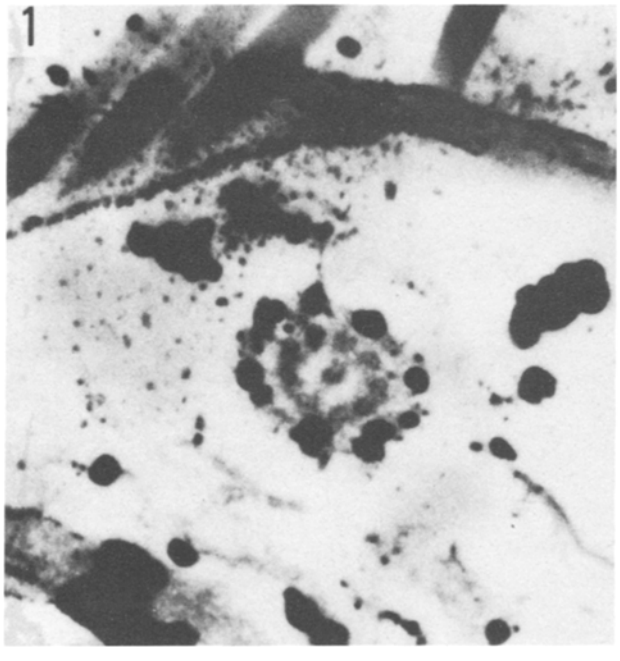

Figure 1. Tangential section through the cell surface of *Paramecium caudatum*. Deposits are seen at the inner side of the ciliary membrane. The irregularly shaped deposits around a cilium are extracellular ones. Weakly stained with uranyl acetate and lead citrate. × 60,000.

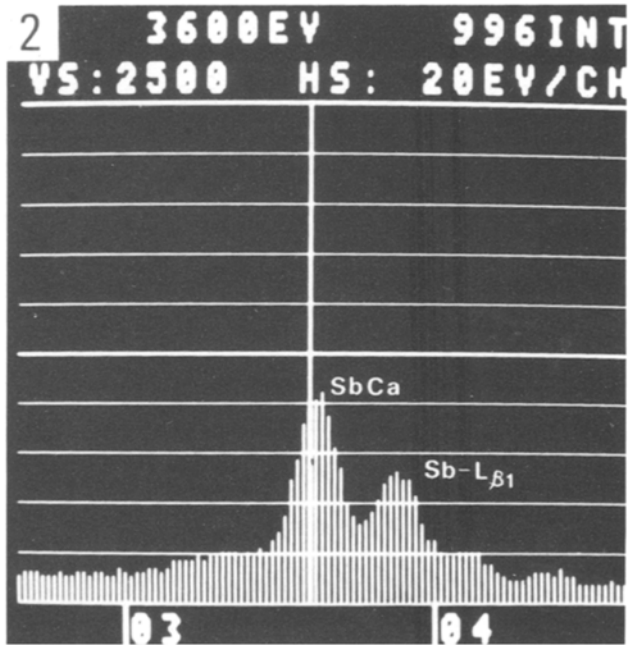

Figure 2. X-ray energy spectrum obtained at the intracellular deposit. Note the most distinct peak at 3620-40 eV; the combined peak of Sb-Lβ, emission (3840 eV) is also seen. Vertical white line indicates the position of Sb-Lα.

The present experiments were undertaken to show the presence of the Ca-binding sites in *Paramecium* by the use of a fixative which did not contain Ca ions, i.e. the pyroantimonate fixation method.

Specimens of *P. caudatum* grown in hay infusion were washed with the adaptation solution, 2 mM $CaCl_2$, 1 mM Tris-HCl pH 7.2, and were adapted for more than 30 minutes before fixation. They were fixed for one hour at room temperature in 1% OsO_4 solution, pH 6.8 adjusted by 0.01 N acetic acid, containing 2% potassium pyroantimonate ($K_2H_2Sb_2O_7 \cdot 4H_2O$). In fixation, special care had to be taken to make the volume of the adaptation solution as small as possible, 0.02 ml or less, and a large volume of fixative, 10 ml or more, was added to the adaptation solution which contained the specimen, otherwise no deposits could be observed within the cell. The specimens were dehydrated in ethanol, and embedded in epon 812. Ultrathin sections, cut on a Porter-Blum MT 2 microtome, unstained or stained weakly with uranyl acetate and lead citrate, were examined with a Hitachi HS-9 electron microscope. Carbon was evaporated on the section for X-ray microanalysis, which was made with an energy dispersive X-ray microanalyzer (EDAX 707B) attached to a Hitachi HHS-2R scanning electron microscope.

Highly electron-dense deposits were found on the inner surface of the ciliary membrane above the axosome, in almost all the cilia, and this region is the same one as observed in the glutaraldehyde method[6]. However, there were some differences in results between the 2 methods. In the present method, a lot of irregular shaped deposits were observed in the extracellular space (fig. 1). Intracellular deposits at the bases of cilia appeared nearly round and their diameter was commonly 30–50 nm, which was similar to their size when observed using the glutaraldehyde method. No deposits were present in the cilia of the specimens treated with a Ca-free solution before fixation. Since pyroantimonate is known to produce electron-opaque precipitates not only with Ca but also with other cations, the presence of Ca in the deposit was examined by means of the electron probe X-ray microanalysis. A typical result of the spot analysis performed on the deposit at the bases of the cilia is shown in figure 2. The X-ray spectrum shows a distinct peak at 3620-40 eV, which is a combination of Sb-Lα emission (3600 eV) and Ca-Kα emission (3690 eV). This fact indicates that the deposit contains Ca[9,10]. The peak of Sb-Lβ, emission (3840 eV) is also seen.

In view of the fact that the intracellular deposits could be observed only when a large volume of the fixative was used, it is considered that most of the extracellular Ca ions in the adaptation solution combined with $H_2Sb_2O_7^{--}$ to form $CaH_2Sb_2O_7$ which could not penetrate the cell membrane, and only $H_2Sb_2O_7^{--}$ could penetrate and bind to intracellular Ca.

Although the role of these binding sites is not clear, the localization of the deposits just above the ciliary necklace[11] linking the ciliary membrane and the outer doublet microtubules suggests the important role of these binding sites in the control of the direction of ciliary beating. Recent results show that Ca ions do not change the direction and the velocity of the sliding of the microtubules obtained from *Paramecium* cilia[12]. This fact indicates that Ca ions do not act directly on dynein but on other structures which are destroyed by the trypsin treatment. Therefore, what we have to find is the sites of action of Ca ions in a cilium and after that the function of the Ca-binding sites, shown in the present and preceding work[6,7], will be clear.

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Aging of the erythrocyte. II. Activities of peroxide-detoxifying enzymes

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Summary. The activity of glutathione peroxidase decreases and that of catalase does not change during erythrocyte aging, as judged from a comparison of bovine red cell fractions separated by density.

A general phenomenon associated with red cell aging in vivo is a decrease in the activities of a majority of erythrocyte enzymes[2–4]. In some cases these changes seem to be of diagnostic value only but others may have serious consequences for the further functioning of the cell[3,5]. Alterations in the activities of defensive enzymes of oxygen metabolism might belong to the 2nd group, as these enzymes seem to be indispensable for the protection of the cell against deleterious reactions of active oxygen intermediates[6]. We have demonstrated that erythrocyte aging involves a decrease in superoxide dismutase activity[7]. The present report deals with the behavior of 2 other enzymes of this protective pathway; catalase (hydrogen-peroxide: hydrogen-peroxide oxidoreductase, EC 1.11.1.6) and glutathione peroxidase (glutathione: hydrogen-peroxide oxidoreductase, EC 1.11.1.9), during the aging of bovine erythrocyte.

Material and methods. Bovine erythrocytes were separated according to age by the method of Murphy[8]. The ultracentrifuged suspension was separated into 6 equal-volume fractions. Glutathione peroxidase activity was estimated according to Sinet et al.[9]. Catalase activity was determined by the method of Beers and Sizer[10]. Hemoglobin was assayed according to Drabkin[11]. Erythrocyte density was estimated by the dropping time method[12].

Results and discussion. Mean density of erythrocytes in successive fractions withdrawn from the top of centrifuge tubes increased progressively from 1.081 (fraction 1) to 1.090 (fraction 6) on the average. The density of bovine red cells has been shown to correlate with their physiological age[13]. Therefore separation by density apparently produces fractionation of erythrocytes according to age. This conclusion is confirmed by distribution of ^{59}Fe-labeled cells of different age in the stratified column of bovine red cells