co-workers (*31*) which demonstrated that defective Rous sarcoma virus particles synthesized in chicken factor–negative cells had no detectable gp85 or gp35 by analysis on polyacrylamide gels. However, it could not be excluded that very small amounts of the glycoprotein or a portion of the molecules with markedly different electrophoretic properties, possibly coded by fraction of undeleted envelope genetic material, may have been present. In this context, studies (*32–34*) strongly imply a stringent requirement for recognition by the viral core proteins of homologous envelope components during formation of pseudotypes of vesicular stomatitis and murine leukemia viruses. Interestingly, the studies by Witte and Baltimore (*32*) indicate that it is sufficient for the glycoproteins to be present in very small amounts for the assembly process to occur.

Although we have restricted our discussion to type C oncornaviruses, the recently established (*35*) precursor-product relationship between mouse mammary tumor virus type A particles and mature type B particles suggests that this class of oncornaviruses may assemble by mechanisms similar to those proposed for type C particles.

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Costae of *Tritrichomonas foetus*: Purification and Chemical Composition

Abstract. *The costa is an intracellular organelle common to all trichomonads. Costae from* Tritrichomonas foetus *have been purified by a method which involves lysis of* T. foetus *with the heat-stable hemolysin produced by* Pseudomonas aeruginosa, *followed by differential centrifugation. Analysis of the purified costae demonstrated that the organelle is composed of 95 percent carbohydrate and 5 percent protein. The carbohydrate moiety, probably a polysaccharide, consisted of glucose (95 percent), mannose (0.4 percent), glucosamine (1.4 percent), ribose (0.6 percent), and an unidentified sugar (2.6 percent). The kinetosomal complex was attached to the costa after initial lysis of cells but was separated from the costa during purification.*

Protozoa of the family Trichomonadidae are of particular interest because some members, *Tritrichomonas foetus* and *Trichomonas vaginalis*, are transmitted by a venereal mode to cattle and humans, respectively. The ultrastructure of the organism is complex and has been studied in detail by both light and electron microscopy (*1*). However, none of the observed organelles have been purified so that their chemical composition can be determined, a prerequisite to understanding structural and functional relationships.

We have developed a procedure for purification of the costa, a supportive organelle common to all trichomonads, and have determined its chemical composition. In addition, we have obtained evidence of a structural association among costae, kinetosomes, and flagella.

In our research we employed the parasite of bovines, *Tritrichomonas foetus*. The culture of *T. foetus* was isolated from an infected bull in a herd located near Baton Rouge, Louisiana. The culture was maintained in NIH thioglycollate broth (Difco Laboratories, Detroit) amended with 5 percent filter-sterilized, heat-inactivated bovine serum. To grow large quantities of cells, liter quantities of the same medium in screw-cap erlenmeyer flasks were inoculated with a 10 percent volume of a mature culture. The cultures were incubated for 18 to 20 hours at 37°C. The cells were harvested by centrifugation and washed twice in 0.85 percent NaCl. For purification of costae, 1.0 ml of packed washed cells was diluted with 3.0 ml of saline, and the cells were lysed by the addition of 800 units of purified heat-stable hemolysin produced by *Pseudomonas aeruginosa* (*2, 3*) in a volume of 1.0 ml. After lysis of the trichomonads, 1.0 mg each of phospholipase C, deoxyribonuclease, and ri-

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bonuclease was added to 5 ml of lysate, and the mixture was incubated at 37°C for 2 hours. The mixture was agitated occasionally to facilitate removal of flagella and associated structures from the costae.

Differential centrifugation was employed to purify the costae. The pellet obtained between sedimentations at 1500 and 6000*g*, with 10-minute centrifugation times, contained the bulk of the free costae. This fraction was suspended in saline and the differential centrifugation was repeated four times. The purified costae were suspended in saline and stored at 4°C for electron microscopy. Purified costae appeared intact after storage for 14 days. For dry-weight determinations and chemical analysis, the costae were washed twice with and finally suspended in glass-distilled water.

To identify fractions containing costae in developing the purification scheme, the costal preparations were visualized by light microscopy of heat-fixed smears stained with crystal violet. To check for purity of the preparation, costae were negatively stained with 0.1 percent phosphotungstate or ammonium molybdate and viewed with an electron microscope.

The purified costae were quantitatively assayed for protein by the method of Lowry *et al.* (*4*) with bovine serum albumin as a standard. Carbohydrate was determined by the phenol sulfuric acid method (*5*), using glucose as the standard.

The sugars constituting the carbohydrate component of the costa were identified by gas-liquid chromatography (GLC). Costae were hydrolyzed with 2*N* HCl at 100°C for 2 hours. The hydrolyzate was neutralized with 2*N* NaOH, and silyl derivatives or alditol acetates, as well as standard sugars, were prepared before analysis (*6*). Two 6-foot glass columns packed with either 3 percent OV-210 or 3 percent OV-17 on Chromosorb W (HP) (Pierce Chemical Company, Rockford, Illinois) were employed for identification, using programmed runs from 150° to 210°C.

The costal organelle extends nearly the entire length of the trichomonad cell and is located beneath and parallel to the undulating membrane (Fig. 1A). Figure 1B is an electron micrograph of costae as they appear after lysis of *T. foetus* with hemolysin and staining with phosphotungstate. The association of the kinetosomes and the four flagella near the anterior end of the costa is apparent. The associated kinetosomal complex can be separated from costae by treatment with phospholipase C and mild agitation. The association between costae, kinetosomes, and flagella may be necessary for motility. Figure 1C is an electron micrograph of phosphotungstate-stained purified costae. There is little evidence of contaminating material, and the integrity of the costae does not appear to be altered.

Analysis of 7.2 mg (dry weight) of purified costae showed 6.9 mg of carbohydrate (about 95 percent) and 0.4 mg of protein, which in sum is somewhat greater (101.4 percent) than the determined dry weight. No lipid material was detected in chloroform-methanol extracts of

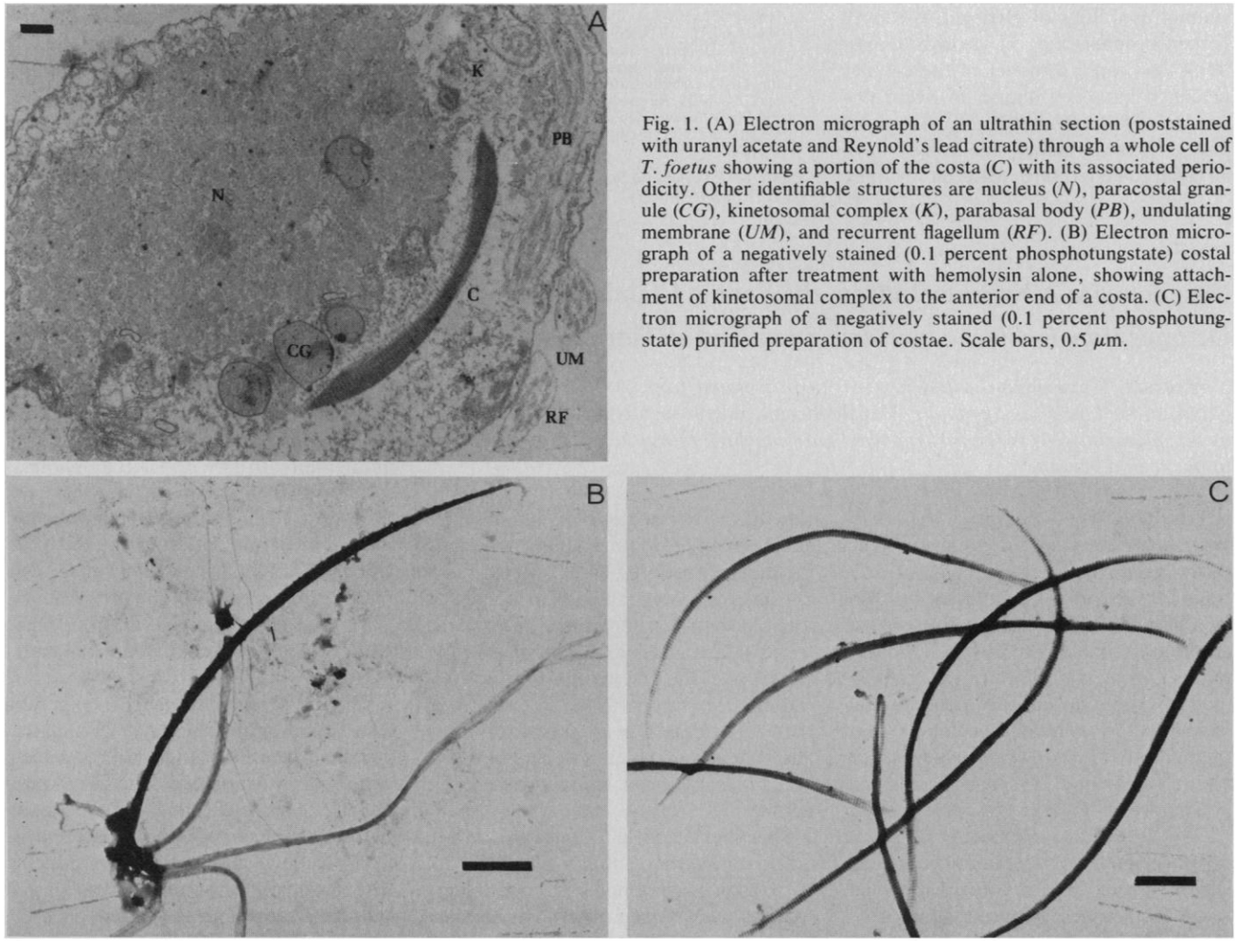

Fig. 1. (A) Electron micrograph of an ultrathin section (poststained with uranyl acetate and Reynold's lead citrate) through a whole cell of *T. foetus* showing a portion of the costa (*C*) with its associated periodicity. Other identifiable structures are nucleus (*N*), paracostal granule (*CG*), kinetosomal complex (*K*), parabasal body (*PB*), undulating membrane (*UM*), and recurrent flagellum (*RF*). (B) Electron micrograph of a negatively stained (0.1 percent phosphotungstate) costal preparation after treatment with hemolysin alone, showing attachment of kinetosomal complex to the anterior end of a costa. (C) Electron micrograph of a negatively stained (0.1 percent phosphotungstate) purified preparation of costae. Scale bars, 0.5 μm.

costae assayed by thin-layer chromotography. If lipids were a component of the costa, they may have been removed by the hemolysin or the phospholipase treatment, or both, and must not have a role in structural integrity.

The GLC analysis of purified costae showed the carbohydrate component to be 95 percent glucose, 1.4 percent glucosamine, 0.6 percent ribose, 0.4 percent mannose, and 2.6 percent an unidentified moiety. Glucose is the main component, and whether the sugars found in small amounts are part of the costa or are derived from contaminating material is conjectural.

Protein (about 5 percent) is a vital component of the structure because exposure of purified costae to trypsin resulted in disintegration and apparent solubilization of the entire structure. Trypsin has been reported to destroy costae in trichomonads (*7*), which implied a protein component of costae. Also, it has been hypothesized that costae are composed of collagen-like material because of their striated appearance (*8*), even though collagenase did not affect costal integrity (*7*).

Tritrichomonas foetus contains substantial quantities of glycogen (10 to 30 percent) depending on growth conditions, and rapid turnover of the cellular glycogen has been shown to occur (*9*). Because of the high glucose content of purified costae, the organelle may have an energy-generating function or may serve as an energy reserve, in addition to the structural function served by its rigidity. The structural and other functions of costae require reconsideration in light of our findings of the chemical composition of this organelle in the purified state.

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Maculotoxin: A Neurotoxin from the Venom Glands of the Octopus *Hapalochlaena maculosa* Identified as Tetrodotoxin

Abstract. *Maculotoxin, a potent neurotoxin isolated from the posterior salivary glands of the blue-ringed octopus,* Hapalochlaena maculosa, *has now been identified as tetrodotoxin. This is the first reported case in which tetrodotoxin has been found to occur in a venom.*

The blue-ringed octopus, *Hapalochlaena maculosa*, is a small octopus that is common along the coast of Australia. It derives its popular name from its ability to display brilliant blue rings of color on its skin when disturbed. After a number of human fatalities (*1, 2*) attributed to the bite of this octopus, the chemistry (*3, 4*) and pharmacology (*5*) of extracts of its posterior salivary glands were investigated. The pharmacological action of maculotoxin, the principal neurotoxin present in extracts of these venom glands, was described (*6*) as being similar to that of tetrodotoxin, although some differences between them have been noted (*7*). We now report the isolation of pure maculotoxin in quantities sufficient to yield information concerning its chemical nature. Direct spectral and chromatographic comparison of maculotoxin with tetrodotoxin shows them to be indistinguishable. *Hapalochlaena maculosa* is the first species in which tetrodotoxin has been found in extracts of the venom glands, in contrast to all other known cases in which it occurs as a poison in the skin, muscle, liver, ovaries, or eggs (*8, 9*).

Specimens (250) of *H. maculosa* were collected in early autumn off the South Australian coast. Upon collection the octopuses were frozen. The posterior salivary glands were removed by dissection and homogenized with 300 ml of 3 percent acetic acid. The homogenate was centrifuged at 16,000*g* for 0.5 hour, and the supernatant was decanted, frozen, and dried. The residue was extracted four times with 3 percent acetic acid. The combined material extracted by aqueous acetic acid weighed 13.4 g and had an activity of 2 mouse units (*8*) per milligram (M.U./mg). The extract was dissolved in 500 ml of 3 percent acetic acid and concentrated to 100 ml by passage through an Amicon Diaflo UM2 ultrafilter (retention > 1000 daltons). The concentrate was filtered by dialysis at a constant volume with 1 liter of acetic acid. This yielded 6.3 g of filtrate having an activity of 3 M.U./mg; 7.0 g of inactive material was retained by the filter.

The filtrate was dissolved in 15 ml of 0.1*M* ammonium acetate at *p*H 6.0 and applied to a column (2.5 by 40 cm) of CM-Sephadex C-25 gel (ammonium form). The column was eluted with a linear ionic strength gradient of 0.1 to 0.4*M* ammonium acetate at *p*H 6.0. The eluted lethal fractions were combined and freeze-dried; they afforded 179 mg of material having an activity of 70 M.U./mg. Only one group of lethal fractions was observed even after elution of the column with five void volumes of 3 percent acetic acid. Savage and Howden (*4*), however, appeared to have detected two toxins when the isolation was carried out by a different method. The lethal material (179 mg) obtained from the initial ion-exchange chromatogram was rechromatographed three times on a CM-Sephadex C-25 column under the same conditions as before. This afforded 1.8 mg of pure maculotoxin having an activity of approximately 7000 M.U./mg. Tetrodotoxin from Sigma Chemical Company had comparable toxicity.

A 30-mg sample of the diafiltrate was subjected to isoelectric focusing, at a *p*H gradient of 7 to 11 with LKB ampholines in a support gel of superfine Sephadex G-75 on an LKB Multiphor instrument. An initial current of 20 ma at 400 volts was applied for 20 hours; the voltage was then increased to 1000 volts for 8 hours. Only one band having an isoelectric point of 8.5 ± 0.1 contained lethal material.

The purification of maculotoxin was also monitored by thin-layer chromatography on Merck silica gel GF 254 plates, which were developed with an isopropanol, acetic acid and water system (14:1:5). Maculotoxin was detected as a light yellowish-brown spot by spraying the plate with a mixture of vanillin and sulfuric acid, and then heating to 110°C, or as a yellow spot under long-wave-

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