the non-ovulatory or in the ovulatory period (fig. 5); the dye hesitated to pass the ostium tubae and a slow and sparse transit of the dye particles through the ICS looks as if the dye were carried by simple diffusion.

Discussion. In mice[11], rats[12] and golden hamsters[13] the bursal cavity is completely separated from the peritoneal cavity. During ovulation ova are released into the bursal cavity, travel through the bursal fluid, arrive somewhere on the surface of the ICS and go into the oviduct. As was demonstrated here, the ICS is limited both in position and length for catching ova released away from the ICS. There must be some force that attracts or transports ova to the ICS. Fischel[14] thought that the bursal wall contracts and flushes bursal fluid into the oviduct during ovulation and this flow carries ova. The same mechanism is postulated by Martin et al.[15]; however, those authors also said that they failed to observe such a flow by injection of a dye into the bursal cavity in the peri-ovulatory period in the golden hamster. Blandau's[16] observation is that by ciliary-driven currents ova are attracted to the ostium tubae from ruptured follicles within 5 seconds in rats. Our experiments with dye injection support the result of Martin et al. Mahi-Brown and Yanagimachi[17] demonstrated in vitro that a cumulus cell mass placed in close proximity to or in contact with the ICS is transported into the oviduct, but it is not, if it is put a little away from the ICS. There is no doubt about the presence of focal currents caused by ciliary activity in an adjacent area of the ICS, but their presence does not always mean that they are strong enough to 'attract' such a large cell mass as an ovum-cumulus cell complex far away from the ICS in a convex cavity of the bursa ovarica.

The situation is entirely different, when an ovum-cumulus cell complex is in touch with the ciliated surface of the ICS. As some investigators have demonstrated, electrostatic forces and, most probably, chemical and physical interactions between the ciliary tips and the cumulus matrix may enhance the efficiency of ciliary activity for transporting materials[17-19]. In others words, although ovum-propelling forces generated by the contractile activity of the oviduct should be taken into account, as the contact surface between the ovum-cumulus complex and the ciliary tips increases, the velocity of transit of the complex becomes higher[20]. This means that in the narrow lumen of the oviduct the ovum-cumulus complex is transported more rapidly. Setting aside an alternative discussion of whether contractile activity of the oviductal smooth muscle or ciliary activity of the oviductal epithelium provides the main force to convey the ovum-cumulus complex, the narrow region of the ICS is obviously advantageous for a rapid transport of the complex from the ICS to the ampulla by ciliary activity.

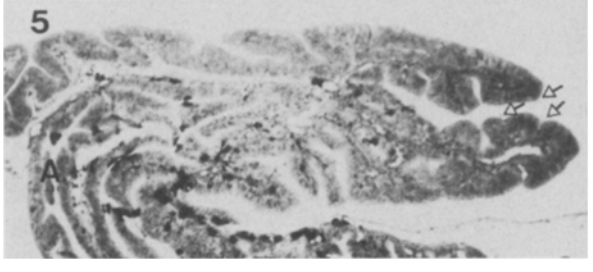

Figure 5. A longitudinal section of the ICS during ovulation. AB particles are seen in the ostium tubae (arrows), but did not go further. ×25.

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Demonstration of the structural connections of the longitudinal muscle cells and circular muscle cells, and interconnections between the two, in the alimentary canal of an oligochaete, *Branchiura sowerbyi* B.

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Summary. The longitudinal and circular muscle cells of the alimentary canal of the oligochaete worm *Branchiura sowerbyi* show intercell couplings. These couplings occur between adjoining cells of the same or of the other orientation.
Key words. Worm, oligochaete; *Branchiura sowerbyi;* oligochaetes; alimentary canal; muscle cells, longitudinal; muscle cells, circular.

Gardy[1] observed antiperistaltic contraction waves of the intestine in the earthworm *Lumbricus terrestris* by means of X-rays, and Naitoh[2] reported several types of contraction waves in the different regions of the alimentary canal of an intact aquatic oligochaete worm *Branchiura sowerbyi.* Both reports suggest that the normal movements of the alimentary canal of Oligochaeta are propagations of contractions in oral and/or aboral directions from their sites of origin. In the present study, we want to describe the fine structure of the muscle cells of an oligochaete alimentary canal, especially as related to the prop-

agation of the contraction. To our knowledge there have not yet been any reports on the electron microscopy of the alimentary canal of Oligochaeta.

Materials and methods. Studies were made on a freshwater oligochaete *Branchiura sowerbyi* B., ranging between 4.5 and 5.0 cm in length. For electron microscopy, the worms were fixed with 1.5% glutaraldehyde and 1% osmium tetroxide. Then specimens were dehydrated by alcohol, embedded in epoxy resin, and cut into silver-gold sections in a transverse or lengthwise direction with a L.K.B. ultramicrotome. Sections were double stained with uranyl acetate and lead citrate, and observed with a JEM-100C electron microscope. The alimentary canal of the worm is divided into several regions[2]; buccal cavity, anterior-and posterior-esophagus, and fore-, mid- and hind-intestine. Observations were chiefly made on the mid-intestine.

Results. Epithelial cells with cilia covered the luminal surface of the alimentary canal. Two muscle layers, inner circular and outer longitudinal, lay below the epithelial layer. The outer surface of the alimentary canal was covered with chloragogen cells. Connective tissue was rarely developed between these layers. Blood sinus stood out particularly between the epithelial layer and the muscle layer. We carefully studied 146 serial transverse sections in one specimen and more than 1000 transverse sections prepared randomly from several worms. But no somas nor axons were detected in the muscle layer of the mid-intestine; this is a difference from vertebrates, in which Auerbach's plexus is seen.

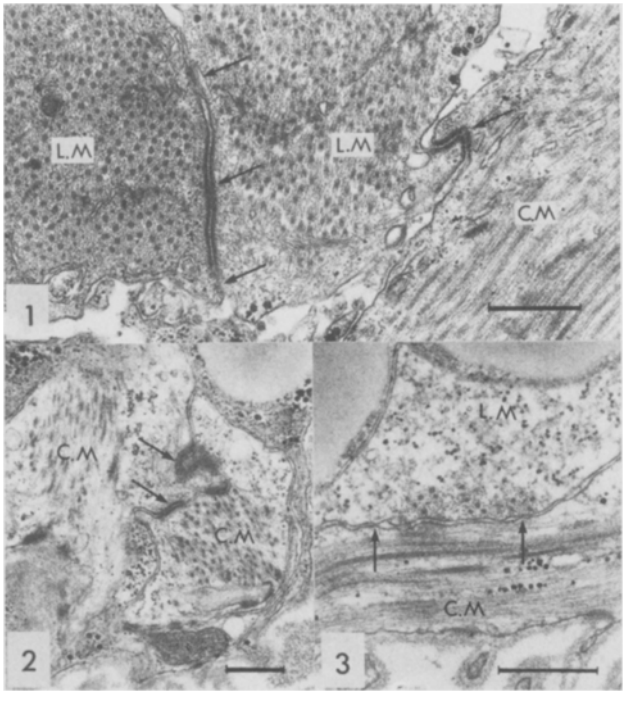
Both the longitudinal and circular muscle layers of the alimentary canal were monocellular layers in which muscle cells were coarsely scattered at an appreciable distance though they lined up more densely in the posterior esophagus than in the rest. The form of the muscle cell was an elongated spindle shape with an outward protuberance in the middle portion of the cell, where a single nucleus was placed. Myofibrils exhibited an intermediate pattern between well-developed oblique-striated muscles and ordinary smooth muscles. Two adjoining muscle cells in both longitudinal and circular layers were directly connected with each other at the tapering end regions of the cells by rigid structures of cell contacts.

The most important finding in the present study was that neighboring longitudinal and circular muscle cells were also coupled together with the same cell connections as the longitudinal muscle cells and circular muscle cells themselves. There were two types of cell-contacts. One was an intercalated disk-like structure similar to the cell-connections of vertebrate cardiac muscles[3] (figs 1 and 2). One or both sides of waving desmosome-like structures, that is electron-dense plaques of membranes, were usually accompanied by a blurred image of membranes (fig. 1), though a typical nexal structure[4] was not observed. The other type was a cell-connection resembling the tight junction[4] (fig. 3). Two membranes were closely apposed in a spot-like manner. This type was less numerous than an intercalated disk-like type.

Discussion. In the present worm, there exist both descending and ascending contraction waves in the esophagus, and ascending contraction waves and defecation movements in the intestine except for irregular or weak movements in the empty alimentary canal[2]. When tetrodotoxin, a blocker of sodium channels and hence of action potentials in the membranes of excitable cells[5], is administered, the worm is soon paralyzed, but rhythmic ascending contraction waves of the mid-intestine continue as well as some other movements[2]. This result implies the possibility that, in addition to the myogenic nature of the movements, contraction propagates through non-neural pathways.

In the present study, we found that muscle cells of the alimentary canal of *B. sowerbyi* were, irrespective of their orientations, directly connected by cell contacts of an intercalated disk-like type accompanied by a possible fusion of membranes, and of the tight junction type. Aside from the transmission of tension, if the excitations propagate electrically from cell to cell, the sites connected by the cell contacts can reasonably be predicted to be the nonpolarized pathways of the propagation of excitation, because coupling sites are the only places where myofibrils of longitudinal and circular layers, which are in a lattice arrangement, can be connected with each other. Direct cell-to-cell propagation of excitation may fit the results with tetrodotoxin mentioned above. The functional difference between the two types of cell contracts is unknown. But one or both of them are assumed to be electrically low-resistance pathways for current flow as is the intercalated disks of vertebrate cardiac muscles[6] and nexus[7] or close contracts of cell membranes[8,9] in the vertebrate visceral smooth muscles. When the excitation propagates between longitudinal muscle cells and circular muscle cells, it is deduced that the contraction of the longitudinal muscle cells is largely responsible for the velocity of the moving speed of contraction, and that of the circular muscle cells is responsible for the propulsive force of contents. In the small intestine of guinea pigs, Hukuhara and Fukuda[10] insisted that both longitudinal and circular muscle layers contract simultaneously. This seems to harmonize well with the present study.

Gabella[8] reported rare but direct connections between longitudinal and circular muscles in the ileum of guinea pigs. On the other hand, Taylor et al.[11] observed a bridge of connective tissue cells between the two muscle layers of cat small intestine and suggested that this bridge electrically coupled the muscle layers. Except for their papers, no publications are available on the mechanism of the propagation of excitation between the longitudinal and circular muscle cells in the alimentary canal



Figures 1–3. Electronmicrographs showing cell contact structures between the muscle cells in the mid-intestine of an oligochaete *Branchiura sowerbyi*. L.M, longitudinal muscle cell; C.M, circular muscle cell. Arrows indicate the cell contact structures. Intercalated disk-like type is shown between L.Ms and between L.M and C.M in figure 1, × 24,000, and between C.Ms in figure 2, × 16,000. Intercalated disk-like cell-contact between L.Ms in figure 1 is accompanied by possible fusion of the plasma membranes (top and bottom arrows). Connections of the tight junction type between L.M and C.M are shown in figure 3, × 26,000. Scale is 0.5 μm in each figure.

of vertebrates. It appears that the mechanisms for the propagation of contractions in the alimentary canal in *B. sowerbyi* and vertebrates may be analogous. From a comparative standpoint, some parts of the direct communications between the longitudinal and circular muscle layers in the relatively simple alimentary canal of the worm described here may be replaced by the connective tissue cell bridge in the highly developed alimentary canal of mammals.

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Enhanced expression of Epstein-Barr virus early antigens by antitubulin agents in a latently infected human lymphoblastoid cell line[1]

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Summary. 28 anticancer agents have been surveyed for Epstein-Barr virus (EBV) activating potency. Two vinca alkaloids with antitubulin activity, vinblastine (VLB) and vincristine (VCR), enhanced the expression of EBV early antigens (EA) in a latently infected human lymphoblastoid cell line (Raji), when used in combination with *n*-butyrate. Other antitubulin agents, colchicine, colcemid, and podophyllotoxin, had the same effect, although their effects were less than that of the potent tumor promoter, 12-O-tetradecanoyl phorbol-13-acetate (TPA).
Key words. Epstein-Barr virus; tumor promoter; antitubulin agents; vinblastine; vincristine; anticancer agents.

The idea that neoplastic development consists of at least two qualitatively different stages has gained general acceptance. The second, promotion stage has been suggested to be more critical in the development of human cancer than the first, initiation stage (reviewed in Sugimura[3]). Most tumor promoters do not bind DNA and are negative in the Ames mutagenicity assay, whereas initiating carcinogens usually meet one or both of these criteria. Recently, TPA and only those other phorbol esters with tumor promoting activity have been found to induce the viral cycle in latently infected EBV genome carrying cells[4], and we have suggested that a system consisting of EBV nonproducer Raji cells and a low concentration of *n*-butyrate might be useful as a practical screening method for certain types of tumor promoter in our environment[5]. Since many widely used anticancer agents, such as chlorambucil, cyclophosphamide, and procarbazine are carcinogenic in experimental animals, and some are possibly tumor promoters[6], we surveyed 28 anticancer agents for the EBV-EA inducing activity and found enhancement of their expression by two vinca alkaloids as well as other antitubulin agents.

Materials and methods. The human lymphoblastoid cell line Raji, carrying the EBV genome was cultivated in RPMI 1640 medium as described previously[5]. The spontaneous induction rate of EBV early antigens in our subline was less than 0.01%. To assure optimal conditions during the experiment, the viability of the cells was checked both before and after treatment, using the dye exclusion test.

The sources of anticancer agents included Shionogi Co. and Takeda Chemical Indust., Osaka; Nihon Shinyaku, Kyoto; Bristol-Banyu Pharm. Co., Chugai Pharm. Co., Kyorin Yakuhin, Kyowa Hakko Kogyo, Meiji Seika, Nihon Kayaku, Nihon Roche, Sankyo, Taiho Yakuhin Kogyo, and Yamanouchi Pharm. Co., Tokyo, Japan. TPA was obtained from Chemical Carcinogenesis Inc., USA. The *n*-butyrate, colchicine and colcemid were purchased from Nakarai Chemicals Kyoto, Japan. Griseofulvin and γ-lumicolchicine were from Sigma Chemical Co., and podophyllotoxin was from Aldrich Chemical Co., Inc., USA.

The test substances were dissolved either in water, ethanol or dimethyl sulfoxide and assayed for EBV-EA inducing activity as previously described[5]. Briefly, Raji cells were incubated for 48 h with both 4 mM *n*-butyrate and the test substance, and

Table 1. EBV-activating potency of anticancer agents

Anticancer agent*	EBV-EA positive, cells (%)**	Anticancer agent*	EBV-EA positive cells (%)**
Actinomycin	1.2	GE132	1.2
Adriamycin	0.1	Methotrexate	0.8
5-Azacytidine	1.2	Mithramycin	1.0
Bleomycin	1.2	Mitomycin	0.7
Busulfan	0.5	Neocarzinostatin	1.2
Chlorambucil	0.5	Nimustine	
Chromomycin A3	0.5	hydrochloride	1.0
Cis-platinum		OK432	1.2
diammine dichloride	1.2	Pepromycin	1.2
Cytarabine	0.5	PSK	1.2
Daunomycin	1.3	Streptozotocin	1.2
Diethylstilbestrol		Vinblastine	12.0
diphosphate	1.4	Vincristine	13.1
Ftrafur	1.2	VP-16	1.0
5-Fluorouracil	1.2	Control***	2.0

* The anticancer agents were tested at the concentrations of 0.1, 1, 10, 100 and 500 μg/ml. ** Raji cells were incubated with 4 mM *n*-butyrate and various concentrations of test substance for 48 h. Only the maximum values observed for each agent are shown. Values are means of at least two cultures. *** 4 mM *n*-butyrate alone. Values were mostly between 0.1 and 0.2.