presence of intranuclear paramyxovirus-like particles in mononuclear cells infiltrating perivascular areas of active myelin breakdown. This has not been observed in other progressive virus diseases of the central nervous system, and it suggests that MS lesions may represent sites of endogenous infection due to periodic seeding of virus-bearing lymphocytes to the central nervous system. However, by analogy with EAE, some special property of the lymphocyte rather than the virus may determine the selective occurrence of lesions in central nervous system white matter. Immunologically specific transformation is required to activate certain latent virus infections of small lymphocytes (*8*); MS may involve a chance latent infection by paramyxoviruses of potentially encephalitogenic lymphocytes, which periodically transform and express virus on contact with central nervous system white matter (*9*).

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9. The patient's circulating lymphocytes, obtained 52 and 72 days before death, were cultured for up to 21 days with fetal calf serum, with and without phytohemagglutinin; electron microscopy of these cells did not reveal virus particles (R. G. Wright, personal communication). For this reason it is suggested that the infection is latent. Recent observations in canine distemper and Marek's disease suggest mechanisms that might be involved in subsequent demyelination in such a situation. H. Wiśniewski, C. S. Raine, W. J. Kay [*Lab. Invest.* **26**, 589 (1972)] described an EAE-like pattern of demyelination by hematogenous cells occurring in the presence of distemper virus in astrocytes. In Marek's disease, circulating lymphoid cells bearing Marek's disease herpes virus in a latent form [J. G. Campbell and G. N. Woode, *J. Med. Microbiol.* **3**, 463 (1970)] incite an EAE-like destruction of myelin in the absence of virus in neural tissue [J. W. Prineas and R. G. Wright, *Lab. Invest.* **26**, 548 (1972)].
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Motility of Macrogamonts of Eimeria magna (Coccidia) in Cell Culture

Abstract. *Amoeboid movement of spheroid or ovoid immature or mature macrogamonts within cultured Madin-Darby bovine cells usually began with the formation of a pseudopodium-like protrusion at the margin of the gamont. The protrusion enlarged as the gamont cytoplasm and nucleus moved into the protruded area. During movement, macrogamonts had an elongate shape. The body of the gamont was constricted as it moved through the cell cytoplasm or from one cell to an adjacent one. After movement had ceased, the gamont resumed its ovoid or spheroidal shape.*

Motility is characteristically present in various stages (sporozoites, merozoites, and microgametes) in the life cycle of *Eimeria* species and related sporozoa. The occurrence of motility has not been reported in other stages of the life cycle, except for the ookinete of malarial parasites (*1*). We recently observed motility in macrogamonts of the rabbit coccidium, *Eimeria magna*, in cell culture.

Monolayers of Madin-Darby bovine kidney cells (120th passage) were inoculated with merozoites obtained by scraping the mucosa of the lower two-thirds of the small intestine of rabbits inoculated 4½ and 5 days earlier with approximately 300,000 oocysts of *E. magna*. The mucosal scrapings were gently stirred with a glass rod, and the mixture was then rinsed in saline A (*2*) containing 5000 μg of dihydrostreptomycin and 5000 units of penicillin G per milliliter. The suspension was centrifuged at 200*g* for 1 minute, and the supernatant fluid, containing the merozoites, was removed. The merozoites were resuspended in minimal essential medium (MEM) in Earle's balanced salt solution without serum or antibiotics. They were counted with a hemacytometer, and 1 ml of MEM, containing approximately 300,000 or 400,000 merozoites, was inoculated into each Leighton tube, with a monolayer on a cover slip (10 by 35 mm). After a 6-hour incubation period at 37°C, 1 ml of MEM containing 5 percent fetal calf serum as well as 50 units of penicillin G per milliliter and 5 μg of dihydrostreptomycin per milliliter was added to each tube. At intervals of 12 hours for a period of 120 hours, cover slips were removed from Leighton tubes and examined in double cover slip preparations (*3*) with phase-contrast microscopy. To record macrogamont motility a filming speed of 120 frames per minute was used with 7242 Ektachrome EF 16-mm film for time-lapse cinemicrography and Tri-X Pan 35-mm film was used for still photomicrographs.

From 36 to 120 hours after inoculation, some intracellular merozoites had developed to micro- and macrogamonts by a process similar to that described previously for *E. magna* (*4*). Simultaneously, other merozoites developed into schizonts with 2 to approximately 500 merozoites. At 36 hours, young macrogamonts were distinguishable from microgamonts by their relatively large size, by the large nucleus and nucleolus, and by the presence of plastic granules in their cytoplasm. At 72 hours, intermediate macrogamonts were ovoid in shape, and 20 live specimens averaged 17 by 12 μm; the nucleus and nucleolus were 6 and 2 μm in diameter, respectively. Mature macrogametes, which were seen at 72 to 120 hours, were similar to the intermediate stages, except that they were larger (27 by 21 μm).

Intracellular movement of young and intermediate macrogamonts was seen at 72 and 84 hours, whereas motile mature macrogamonts were seen at 84 and 96 hours. Usually, the motile macrogamonts moved within the cytoplasm of the host cell and, occasionally, moved into an adjacent cell. During movement, gamonts became elongate. A small pseudopodium-like protrusion developed at the margin of the gamont;

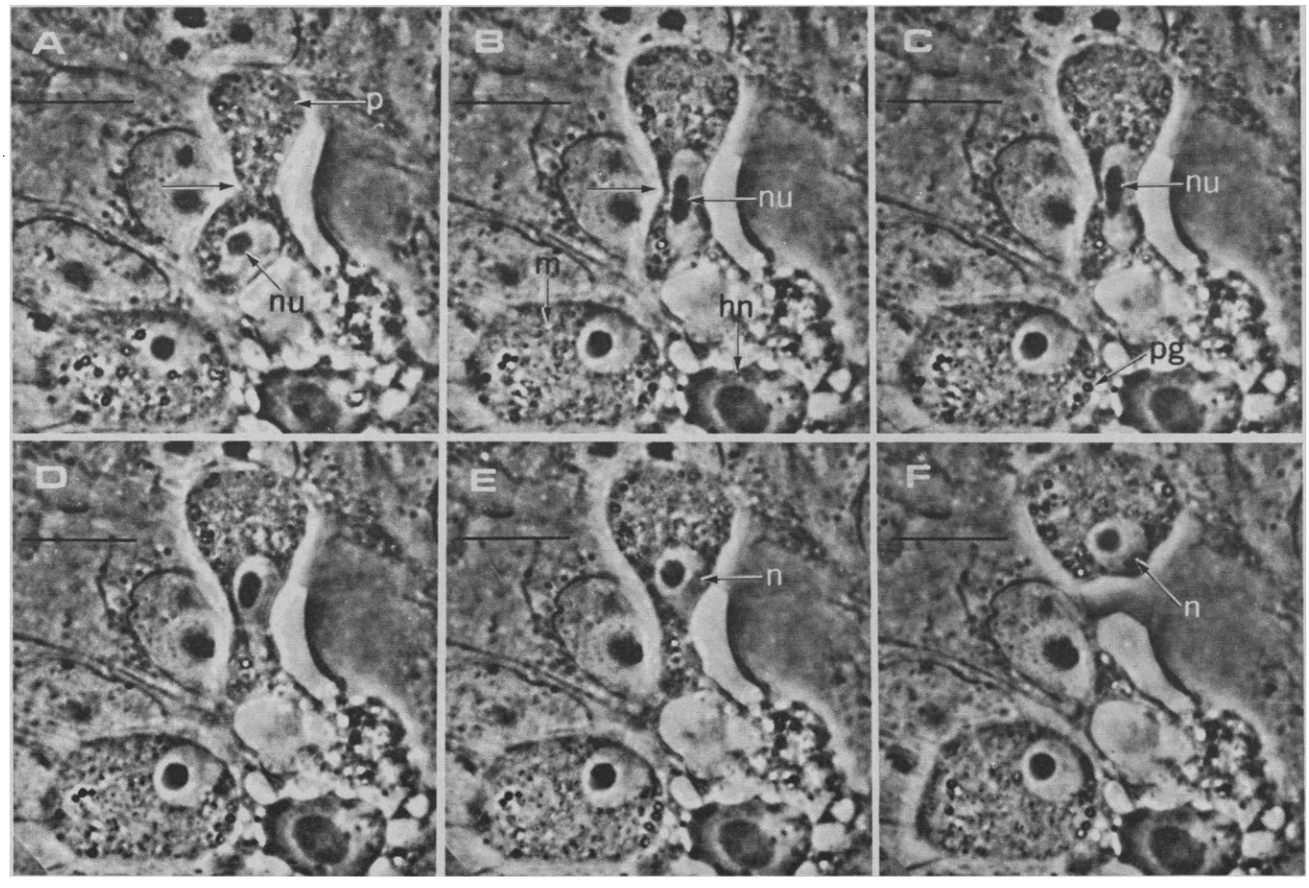

Fig. 1. Photomicrographs of two macrogamonts of *Eimeria magna*, one of which is moving within its host cell; 84-hour Madin-Darby bovine kidney cell culture; phase-contrast microscopy. Abbreviations: *HN*, host cell nucleus; *M*, macrogamont; *N*, nucleus of parasite; *NU*, nucleolus of parasite; *P*, pseudopodium-like protrusion; *PG*, plastic granule. The scale in each photomicrograph is 10 μm. (A) Motile macrogamont with a constriction (arrow) in the middle region of the body and a large pseudopodium-like protrusion. (B) The protrusion of the motile macrogamont is larger; the nucleus and nucleolus have become rod-shaped while passing through the constricted area (arrow). (C) A later stage in intracellular movement of gamont; the nucleolus has changed slightly in shape. (D) The gamont nucleus and nucleolus have started to transform to a spheroidal shape. (E) The gamont has almost completely passed through the constriction (arrow); note the spheroidal nucleus and nucleolus. (F) The spheroidal gamont immediately after movement has stopped.

this gradually increased in length and became wider, especially in the distal region (Fig. 1, A to E). The cytoplasm of the gamont appeared to flow in the direction of the protrusion, with the nucleus eventually moving into the protruded area. In a few specimens, the nucleus moved into the protrusion as movement was beginning. While moving, the macrogamont usually had a constriction at the point of origin of the pseudopodium (Fig. 1A). The nucleus and nucleolus usually became rod shaped as they passed through the constriction (Fig. 1, B and C), after which they assumed their normal spheroidal shape (Fig. 1E). Clear pathways were sometimes left by the movement of a gamont within a host cell. After passing from one cell to another, macrogamonts moved close to the new host cell nucleus. Some indented the nuclear envelope and, occasionally, forced the cell nucleus to one side. Penetration of the host cell nucleus was not seen. After movement ceased, macrogamonts resumed their typical ovoid or spheroid shape (Fig. 1F). Macrogamonts freed from their host cells by removal of the monolayer did not move.

This is the first report of the occurrence of an amoeboid kind of movement in the macrogamonts of coccidia. Sporozoites and merozoites of sporozoa characteristically move by a swimming or gliding process whose mechanism is unknown. Microgametes of many sporozoan species move by flagella. In our study, *E. magna* macrogamonts appeared to move within the cell cytoplasm and from one cell into an adjacent one by amoeboid movement, with a protrusion similar in appearance to a pseudopodium of lobopodium type. Since coccidian macrogamonts have no apical complex, multilayered pellicle, or subpellicular microtubules, which are always present in sporozoites and merozoites, movement in macrogamonts probably occurs by a different mechanism. Fayer (*5*) found that macrogamonts of *Sarcocystis* sp. in cell culture rapidly rotated clockwise and counterclockwise on their longitudinal axis while within the parasitophorous vacuole. Garnham *et al.* (*1*) reported that the ookinete of *Plasmodium* penetrated the epithelial cells of the gut of the mosquito and apparently migrated intracellularly to reach a location at the external border of the gut, where development into the oocyst stage occurred. Since macrogamonts of *Eimeria* species typically complete development into the oocyst without leaving the host cells, the significance of their movement is difficult to explain.

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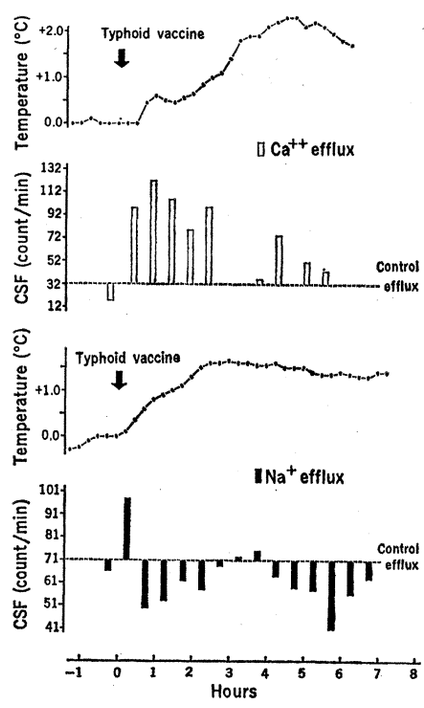
Fever: Reciprocal Shift in Brain Sodium to Calcium Ratio as the Set-Point Temperature Rises

Abstract. *A bacterial pyrogen acts on the brain by disturbing the natural balance between two essential cations in the cerebral region involved in thermoregulation. After typhoid vaccine is administered to the unanesthetized cat, $^{45}Ca^{2+}$ efflux into the third cerebral ventricle increases while $^{22}Na^{+}$ is retained in hypothalamic tissue at the same time that the set-point temperature begins to rise. The subsequent rates of $^{22}Na^{+}$ and $^{45}Ca^{2+}$ efflux parallel the course of the bacterial fever but in a reciprocal fashion. This supports the theory that a change in the set-point temperature is determined by an alteration in the inherent ratio of Na^{+} to Ca^{2+} ions in the hypothalamus.*

An upward shift in the set point for body temperature is a general part of the sequelae associated with a fever of bacterial origin (*1*). Although the cerebral mechanism by which the response to a pathogen is mobilized is unknown, it has been postulated that a pyrogen acts on the posterior hypothalamus to alter the inborn ratio of sodium to calcium ions (*2*). This theory was based on the finding that an intense hyperthermia develops if the endogenous Na^{+} is artificially increased or Ca^{2+} is lowered by a chelating agent in this hypothalamic region. If the theory is correct, either in vivo Ca^{2+} would decline or Na^{+} would increase during a bacterial fever, or both events should occur simultaneously within hypothalamic tissue.

In an anesthetized animal, there is some evidence that a pyrogen may briefly reduce the concentration of Ca^{2+} at some sites in the hypothalamus (*3*) just as systemically administered endotoxin does in serum (*4*). We now report that a reciprocal change in the ratio of the concentrations of endogenous Na^{+} to Ca^{2+} occurs within the brainstem of the conscious cat as a fever develops. Moreover, this ionic ratio parallels the characteristics of the rise in the body temperature of the animal.

In each of three cats, a stainless steel guide tube was implanted aseptically 2 mm above each lateral ventricle according to procedures in (*2*, *5*). After 7 to 10 days, the brain tissue and fluid in the region of the hypothalamus was labeled with either 6 to 8 μc of $^{45}Ca^{2+}$ or of $^{22}Na^{+}$ (New England Nuclear) by injecting the nuclide into the lateral cerebral ventricle. To do this, we connected a length of polyethylene tubing (PE-50) to a 23-gauge, thin-walled injection cannula filled with pyrogen-free, artificial cerebrospinal fluid (CSF) (*6*) containing 0.1 ml of the particular radionuclide. The cannula was then passed through the right guide tube into the ventricle, and after the radioactive solution flowed in, 0.1 ml of additional artificial CSF was infused.



To perfuse the third ventricle of the unanesthetized cat, we modified a procedure used for the monkey (*7*). A 23-gauge, thin-walled, stainless steel cannula was inserted through the guide tube into each lateral ventricle. The right cannula was connected by means of PE-50 tubing to a Harvard variable-speed infusion pump and was used as the inflow, whereas the contralateral cannula served as the outflow (*7*). To collect a sample of CSF, the infusion pump was turned on at a rate of 0.1 ml/min, and the tubing, connected to the left ventricular cannula, was held 5 to 10 cm below the level of the ventricle allowing the CSF to flow out.

The initial portion of the effluent, determined by the volume of nonradioactive CSF in the tubing, was discarded and approximately 0.2 to 0.3 ml of the subsequent perfusate was collected for the determination of radioactivity. A 100-μl portion of each CSF sample was added to a counting vial filled with 15 ml of a scintillation solution containing reagent grade toluene and 2-ethoxyethanol in a 1 to 1 ratio plus 0.8 percent (weight per volume) of scintillation grade 2,5-diphenyloxazole. The samples were then counted for 10 minutes in a Packard 3320 Tri-Carb liquid scintillation spectrometer with automatic external standardization.

During the first 2 hours, two to four samples were collected at 30-minute intervals until the level of radioactivity had stabilized. Then, the inflow cannula was removed and replaced by a similar cannula that was connected to PE-50 tubing filled with a 1 : 4 dilution of *Salmonella typhosa* containing 10^{9} organisms per milliliter (*8*). A volume of 0.2 ml of the diluted pyrogen solution was injected; this amount produces a profound hyperpyrexia in the cat when administered by this route (*9*). Immediately thereafter, the inflow perfusion cannula was inserted again into the right ventricle and CSF samples were again collected at 30-minute intervals throughout the development of the fever.

Fig. 1. Fevers produced by 0.2 ml of diluted typhoid vaccine injected into the lateral cerebral ventricles of two cats at zero hour. Each bar represents a 3- to 4-minute perfusion of the third cerebral ventricle. The efflux of $^{45}Ca^{2+}$ (top) and $^{22}Na^{+}$ (bottom) into CSF is expressed in counts per minute. The value of the sample 1 hour before the typhoid injection served as the baseline (control) after the rate of efflux in each experiment had become stabilized.