proviral DNA was inserted at a new chromosomal site (*7*).

So far, all functional proviral copies carried in the different Mov substrains have been activated during later development (*20*). This activation may depend on the chromosomal position of the proviral DNA (*5, 6*). Thus, retroviral genomes are favorable tools to study mechanisms of gene regulation in embryonic development. The retroviral genomes carried in the Mov-14 substrain are invariably expressed during the lifetime of the animals. The presence of these proviral genomes on the X chromosome should provide a useful marker for studying the molecular events associated with X chromosome inactivation (*21*), since monitoring virus expression does not require the use of the selective methods needed for the study of other X-linked genes (*22, 23*).

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Entamoeba histolytica Causes Intestinal Secretion: Role of Serotonin

Abstract. *Lysates of the protozoan parasite* Entamoeba histolytica *altered active electrolyte transport when present on the serosal surface of rabbit ileum and rat colon. The lysate-induced effects on electrolyte transport were similar to those caused by serotonin, and were blocked by bufotenine, an analog known to inhibit the action of serotonin. The transport effects were partially inhibited by antibody to serotonin. The amebic lysates were shown to contain serotonin by radioimmunoassay, high-performance liquid chromatography, and thin-layer chromatography. These results suggest that the serotonin present in* Entamoeba histolytica *may be important in the diarrhea seen in amebiasis.*

Entamoeba histolytica is a common cause of diarrhea in humans, but the pathophysiology of amebiasis is poorly understood. Lysates of axenically cultivated *E. histolytica* have both cytoxic and enterotoxic activities which may contribute to the pathogenesis of the diarrhea (*1–3*). In the present study, cell-free extracts of *E. histolytica* affected active electrolyte transport when added to the serosal side of rabbit ileum or rat colon. The changes produced were similar to those caused by several neurohumoral substances, including serotonin, which was identified in amebic lysates. During the course of infection, amebas are located in the lamina propria at the bases of intestinal epithelial cells where released neurohumoral substances could contribute to the alteration in intestinal transport to produce diarrhea.

Strains of *E. histolytica* of varying virulence based on animal model criteria were cultured axenically in Diamond's medium (*4, 5*). Trophozoites were harvested in the late log phase of growth, washed three times in phosphate-buffered saline, and resuspended to a final concentration of 10^5 trophozoites per milliliter. A cell-free extract, termed the crude lysate, was obtained by freeze-thawing and sonication. The crude lysate was centrifuged ($10^5 g$ for 1 hour) and a 30 to 70 percent ammonium sulfate precipitate prepared. Previous studies indi-

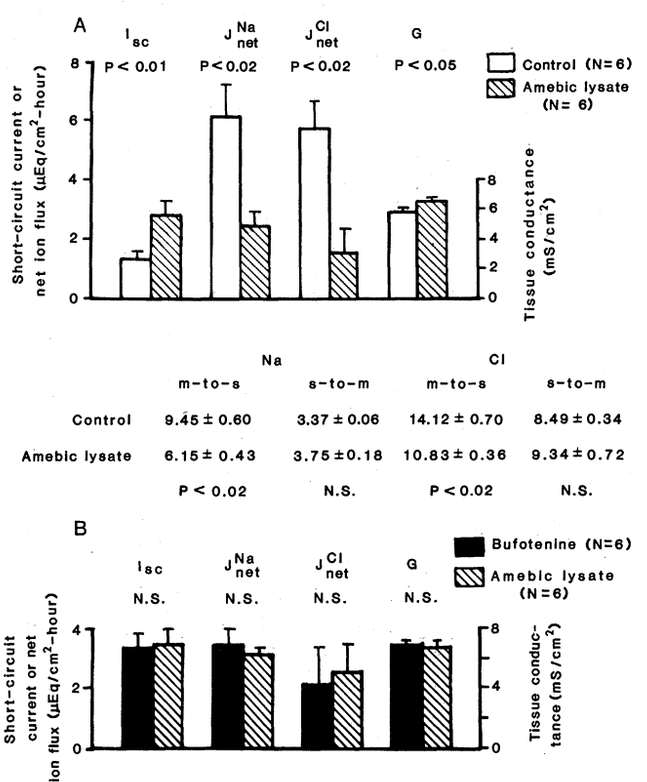

Fig. 1. The effect of *E. histolytica* lysate on rat colonic electrolyte transport. (A) Untreated control tissue. (B) Tissue first exposed to bufotenine. In (A), fluxes were determined in untreated control tissue 20 to 60 minutes after the addition of isotope; amebic lysate was then added and 70 to 110 minutes after isotope addition the effect of the lysate was measured. In (B), bufotenine was added at time 0 and its effect was determined between 20 and 60 minutes after isotope addition; then amebic lysate was added and the fluxes were measured between 70 and 110 minutes. Units of short-circuit current (I_{sc}) and fluxes are microequivalents per square centimeter per hour; conductance (G), millisiemens per square centimeter. Unidirectional (mucosal-to-serosal or serosal-to-mucosal) Na^+ and Cl^- fluxes are shown below (A) to show the effect of lysate. The P values represent comparisons of transport in (A) between untreated control and lysate addition to the same tissues and in (B) between bufotenine and lysate addition to the same tissues (paired t-test). Bufotenine treatment prevented the effect of lysate.

cated that most of the cytotoxic and enterotoxic activities are contained in this latter fraction (*1–3*). Crude lysate and ammonium sulfate precipitates gave similar results in the following studies. Data are presented for crude lysates unless stated otherwise. Control preparations included culture media diluted 1 in 1000 in phosphate-buffered saline to simulate washing of the amebas.

The effects of the amebic lysate preparation on active electrolyte transport were studied by the voltage clamp technique with the use of an Ussing chamber as described (*6, 7*). Rabbit ileal and rat descending colonic mucosa with serosa and muscularis propria removed were mounted as flat sheets in modified Ussing chambers; both the mucosal and serosal surfaces were bathed with solutions of similar ionic composition (Ringer-HCO_3, *p*H 7.4) at 37°C and aerated with 95 percent O_2 and 5 percent CO_2. Electric current was continually passed through the tissue in a direction to reduce the spontaneous potential difference to zero. This amount of current is called the short-circuit current (I_{sc}) and represents the sum of the charge of all electrolyte transport occurring at any time. Exposure of the mucosal surface to amebic lysate had no effect on I_{sc}; this is in contrast to several bacterial enterotoxins which exert their effect from the mucosal surface (*8*). Addition of amebic lysate to the serosal surface of rabbit ileum produced an increase in I_{sc} (Table 1). The response was rapid in onset, peaking within 3 minutes of addition, and transient in nature, returning to baseline within 6 minutes. After return of the I_{sc} to baseline, repeated challenge with the same or larger quantities of amebic extract produced no significant change in I_{sc} (Table 1), suggesting a desensitization phenomenon. The tissue desensitization was completely reversible (Table 1). Tissues were desensitized with amebic extracts, and the serosal bathing solution then was replaced with fresh Ringer-HCO_3 not containing amebic extract. Upon further challenge with amebic lysate, a full response was elicited.

The amebic lysate-induced increase in I_{sc} was anion dependent, calcium dependent, and partially heat-stable (Table 1). The effect was totally prevented by removal of exchangeable anions (Cl^- and HCO_3^- replaced with the nonpermeable anion, isethionate). Since active electrolyte secretion is dependent on uptake of NaCl or $NaHCO_3$ across the basal-lateral membrane of the ileal secretory cell (*9*), this anion dependence suggests that amebic lysates cause active anion secretion. The presence on the serosal surface

Table 1. Effect of *E. histolytica* lysate on short-circuit current (I_{sc}*) in rabbit ileum. Conditions refer to the bathing solution or the addition of amebic lysate to serosal surface.

Condition	Animals (No.)	Maximum increase in I_{sc}* (μAmp/cm^2)	*P*†
Crude lysate	14	20.4 ± 3.3	
Lysate precipitated in 30 to 70 percent ammonium sulfate	3	24.8 ± 9.8	
Heat-inactivated crude lysate	3	12.9 ± 2.8	N.S.
Crude lysate (in Ringer-HCO_3):			
Rechallenge‡	4	4.1 ± 2.4	< 0.05
Washout and rechallenge§	3	16.5 ± 1.3	N.S.
Cl^--free, HCO_3-free Ringer	3	2.4 ± 1.5	< 0.05
Verapamil (10^{-4} *M*)¶	4	3.1 ± 2.6	< 0.02

*Difference in baseline and maximum I_{sc} which occurred within several minutes of adding amebic lysate. †Comparison with response to crude amebic lysate added to Ringer-HCO_3 in tissues from the same animal (paired *t*-test). Diluted medium used as control did not alter I_{sc}. ‡Crude lysate of *E. histolytica* was added to serosal bathing solution after I_{sc} had returned to baseline. §Serosal bathing solution was washed out and replaced with Ringer-HCO_3, and the tissue was allowed to stabilize prior to being challenged. ¶Verapamil was present on the serosal surface 30 minutes before the addition of amebic lysate.

of the Ca^{2+} channel blocker verapamil (Table 1) or a Ca^{2+}-free bathing solution plus 1 m*M* EGTA (data not shown) totally prevented the effect of the amebic lysate on ileal I_{sc}. Strains of *E. histolytica* of varying virulence had similar effects on I_{sc}. Similar to the results with ileum, addition of amebic lysate to the serosal but not mucosal surface of rat colon caused an increase in I_{sc} (Fig. 1). However, the effect in rat colon lasted longer, with I_{sc} remaining elevated for at least 30 minutes. During this time determination of changes in electrolyte transport demonstrated that amebic lysate caused significant and equal decreases in active Na^+ and Cl^- absorption (Fig. 1A).

The amebic lysate–induced changes in electrolyte transport in ileum and colon were similar to those caused by several neurohumoral substances normally present in mammalian intestine, including serotonin (*6, 10, 11*). That serotonin was present in the amebic lysate was demonstrated by radioimmunoassay with a highly specific antibody (*12*), high-performance liquid chromatography with electrochemical detection (*13*), and thin-layer chromatography (*14*) following butanol extraction and ninhydrin derivatization. All *E. histolytica* strains studied contained serotonin as determined by radioimmunoassay (*12*). A virulent strain (HMI) contained 4.1 ng of serotonin per milligram of protein; a moderately virulent strain (200) contained 1.7 ng/mg; and an avirulent strain (Rahman) contained 2.2 ng/mg.

To demonstrate that the serotonin in amebic lysates contributed to the intestinal transport changes, we used a specific antibody to serotonin and the serotonin agonist bufotenine, which blocks serotonin-mediated alterations of ileal and colonic electrolyte transport (*15*). Pretreat-

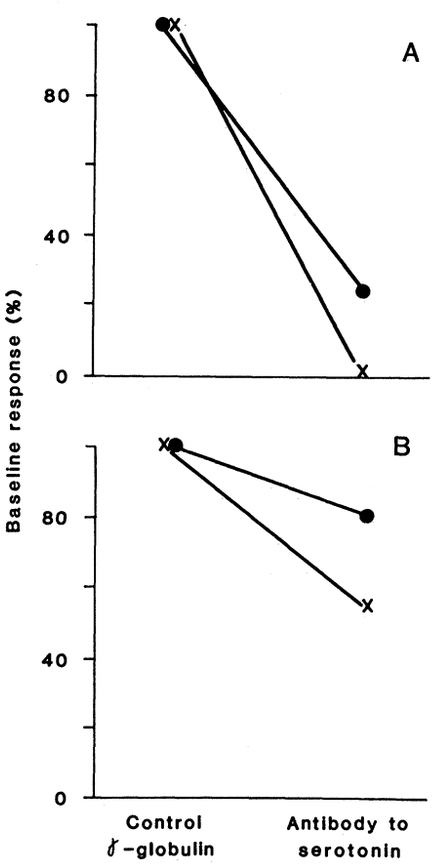

Fig. 2. The effect of antibody to serotonin on (A) the serotonin-induced increase in I_{sc} and (B) the amebic lysate–induced increase in I_{sc}. Sufficient antibody to neutralize ten times the amount of serotonin calculated to be present in amebic lysates by radioimmunoassay or, as a control, an equal volume of γ-globulin from nonimmunized rabbits was added to the serosal surface of rabbit ileum. Ten minutes after the antibody or the control γ-globulin was added, either a known amount of serotonin (A) or amebic lysate (B) was added. The response elicited in two experiments after treatment with control γ-globulin is on the left and that after treatment with antibody to serotonin is on the right.

ment with antibody to serotonin markedly inhibited the serotonin-induced increase in I_{sc} (Fig. 2A). The presence of control γ-globulin from nonimmunized rabbits did not alter the normal maximal serotonin response (Fig. 2). The antibody to serotonin partially inhibited the effect of amebic lysate on ileal I_{sc} (Fig. 2B). Bufotenine decreased net Na^+ and net Cl^- absorption in rat colon (Fig. 1B), effects similar to those caused by serotonin (*16*). After bufotenine, the effect of the amebic lysate was significantly less, but theophylline, another secretagogue, elicited a normal secretory response (data not shown). Thus, serotonin contributes to the amebic lysate-induced transport response but does not explain the entire response.

Hormone-like substances (adrenocorticotropic hormone, β-endorphin, human chorionic gonadotropin, somatostatin, and insulin) and neurotransmitters (catecholamines, serotonin, and acetylcholine) have been detected in bacteria and protozoa (*17–20*); however, their function in unicellular organisms has not been determined. We suggest that the serotonin in *E. histolytica* may play a role in the diarrhea of amebiasis by causing intestinal secretion. The serosal site of action is compatible with the pattern of invasion of *E. histolytica* through the tight junctions of mucosal cells into the lamina propria to form the characteristic flask-shaped ulcerations of amebiasis. The subsequent local release of neurohumoral substances, either by secretion or by lysis of the organism, could lead to a secretory diarrhea since serotonin or other neurohumoral substances present in virulent amebas would be present in a location where they could act in a paracrine fashion to alter active electrolyte transport.

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Malaria Parasites Adopt Host Cell Superoxide Dismutase

Abstract. *Aerobic organisms depend on superoxide dismutase to suppress the formation of dangerous species of activated oxygen. Intraerythrocytic stages of the malaria parasite exist within a highly aerobic environment and cause the generation of increased amounts of activated oxygen.* Plasmodium berghei *in mice was found to derive a substantial amount of superoxide dismutase activity from the host cell cytoplasm. Plasmodia isolated from mouse red cells contained mouse superoxide dismutase, whereas rat-derived parasites contained the rat enzyme. This is believed to be the first example of the acquisition of a host cell enzyme by an intracellular parasite.*

The superoxide anion (O_2^-), a partially reduced form of molecular oxygen, is generated spontaneously and metabolically within biological organisms. Because O_2^- can react to form highly destructive hydroxyl radical (·OH), aerotolerant organisms may need protection against O_2^-. Indeed, almost all known aerobic organisms contain the enzyme superoxide dismutase (SOD) (*1*):

$$2\ O_2^- + 2\ H^+ \xrightarrow{SOD} H_2O_2 + O_2$$

This enzyme may be particularly important for mammalian erythrocytes because activated oxygen species (O_2^-, H_2O_2, and ·OH) form spontaneously within these cells (*2*). Abnormally large amounts of activated oxygen (especially H_2O_2) occur within malaria-infected murine red cells (*3*). One would therefore expect intraerythrocytic forms of malaria to have particularly effective oxidant defense mchanisms.

In view of the probable dependence of the malaria parasite on antioxidant enzymes, we investigated malarial SOD with a view to identifying a new target for the development of chemotherapeutic agents. Accordingly, we measured SOD (*4*) in normal mouse erythrocytes, mouse erythrocytes infected with *Plasmodium berghei*, and isolated parasites (*5*). Contrary to our expectation, malaria-infected erythrocytes showed less SOD activity than normal mouse red cells when expressed on a per cell basis (Table 1). A portion of this decrement undoubtedly reflected parasite-mediated digestion of host cell cytoplasmic contents. Thus, if SOD activity was expressed per unit of protein or hemoglobin, infected cells appeared to have nor-

Table 1. Superoxide dismutase activities of normal and *P. berghei*–infected murine red blood cells (RBC's) and of isolated *P. berghei* (*5*). The results are expressed as means ± standard deviation.

Sample	SOD activity		Percentage inhibition by cyanide ($10^{-3}M$)
	U/10^9 cells	U/mg protein	
Normal RBC's	89.7 ± 12 (N = 8)	4.4 ± 0.5 (N = 8)	85.2 ± 15.5 (N = 5)
Infected RBC's	63.8 ± 16 (N = 10)	6.1 ± 1.5 (N = 10)	
Isolated parasites		12.3 ± 4.1 (N = 10)	82.6 ± 1.1 (N = 3)