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.

KATHERINE MCGOWAN
ANN KANE, NANCY ASARKOF
JOAN WICKS, VICTORIA GUERINA
Department of Medicine,
Tufts University School of Medicine,
Boston, Massachusetts 02111
JOHN KELLUM
Department of Surgery, New England
Medical Center Hospital,
Boston, Massachusetts 02111
SHERRI BARON, ALAN R. GINTZLER
Department of Biochemistry,
Downstate Medical School,
Brooklyn, New York 11203
MARK DONOWITZ
Departments of Medicine and
Physiology, Tufts University Schools of
Medicine, Dental Medicine, and
Veterinary Medicine, and *Sackler*
School of Graduate Biomedical
Sciences, Boston 02111

References and Notes

1. W. B. Lushbaugh, A. B. Kairalla, J. R. Cantey, A. F. Hofbauer, F. E. Pittman, *J. Infect. Dis.* **139**, 9 (1978).
2. K. McGowan, C. F. Deneke, G. M. Thorne, S. L. Gorbach, *ibid.* **146**, 616 (1982).
3. H. J. Bos, *Trans. R. Soc. Trop. Med. Hyg.* **73**, 120 (1979).
4. C. F. T. Mattern and D. B. Keister, *Am. J. Trop. Med. Hyg.* **26**, 402 (1977).
5. L. S. Diamond, D. R. Harlow, C. C. Cunnick, *Trans. R. Soc. Trop. Med. Hyg.* **72**, 431 (1978).
6. M. Donowitz, N. Asarkof, G. Pike, *J. Clin. Invest.* **66**, 341 (1980).
7. M. Donowitz, Y. H. Tai, N. Asarkof, *Am. J. Physiol.* **239**, G463 (1980).
8. M. Field, L. H. Graf, W. J. Laird, P. L. Smith, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 2800 (1978).
9. R. A. Frizzell, M. Field, S. G. Schultz, *Am. J. Physiol.* **286**, F1 (1979).
10. M. W. Walling, T. A. Brasitus, D. V. Kimberg, *Gastroenterology* **73**, 89 (1977).
11. R. J. Miller, J. F. Kachur, M. Field, J. Rivier, "Hormonal regulation of epithelial transport of ions and water," *Ann. N. Y. Acad. Sci.* **372**, 571 (1981).
12. J. M. Kellum and B. M. Jaffe, *Gastroenterology* **70**, 516 (1976). The assay can detect as little as 0.39 pmole (50 pg) serotonin. The antibody was prepared in rabbits and 50 percent inhibition of binding occurred with 5 ng of serotonin. The intra-assay coefficient of variation was 9.4 ± 1.1 percent and the inter-assay coefficient of variation was 9.4 ± 1.7 percent.
13. F. Ponzio and G. Jonsson, *J. Neurochem.* **32**, 129 (1979).
14. M. K. Shellenberger and J. H. Gordon, *Anal. Biochem.* **39**, 356 (1971).
15. M. Donowitz and J. Wicks, unpublished data.
16. K. Dharmsathaphorn, L. Racusen, J. W. Dobbins, *J. Clin. Invest.* **66**, 813 (1980).
17. T. Maruo, H. Cohen, S. J. Segal, S. S. Koide, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 6622 (1979).
18. D. LeRoith, J. Shiloach, J. Roth, M. A. Lesniak, *ibid.* **77**, 6184 (1980).
19. K. Janakidevi, V. C. Dewey, G. W. Kidder, *Arch. Biochem.* **113**, 758 (1966).
20. J. Roth, D. LeRoith, J. Shiloach, J. L. Rosenzweig, M. A. Lesniak, J. Havrankova, *N. Engl. J. Med.* **306**, 523 (1982).
21. We thank S. Gorbach for encouragement and J. Imperato for support. Supported by NIH grant AM26523, RCDA KOH-00588 (M.D.), and a grant from the Houston Oil and Refining Foundation.

23 May 1982

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)

mal or increased amounts of activity (Table 1 and Fig. 1).

Despite the decreased SOD within infected cells, isolated parasites contained substantial SOD activity (Table 1) (*6*). Indeed, in red cells from animals with various percentages of infected cells, there was a concommitant decline in host cell SOD with an increase in parasite-associated SOD activities. At very high parasitemias (90 to 100 percent of cells infected) approximately 20 percent of the total SOD activity was parasite-associated, whereas red cell SOD activity was 20 percent lower (not shown). These results suggested that some host SOD might be taken up by the parasite. Furthermore, although isolated parasites are unavoidably contaminated with host cell cytoplasm, such contamination could not account for the substantial parasite-associated SOD activity because the ratio of SOD to hemoglobin was much higher in the parasite than the host cell (Fig. 1). Indeed, this ratio was more than ten times higher in crude parasite lysates than in uninfected red cells and almost 70 times higher when membrane-associated and particulate hemoglobin was removed by ultracentrifugation (Fig. 1).

To characterize further this parasite-associated SOD, we assessed the inhibitory effects of cyanide. Whereas the mammalian copper- and zinc-containing SOD is cyanide-sensitive (*1*), those of protozoan parasites—such as *Leishmania tropica, Trypanosoma brucei*, and *Trypanosoma cruzi*—have iron at the center and are cyanide-insensitive (*7*). Contrary to our expectation, the SOD associated with malaria parasites was as readily inhibited by cyanide as that of the host cell (Table 1). This cyanide inhibition of plasmodial SOD suggests that the enzyme contains copper and zinc and, therefore, resembles host SOD more than it does protozoan SOD's.

Plasmodial and mouse enzymes are indistinguishable electrophoretically. Isoelectric focussing (IEF) (*7*) and polyacrylamide gel electrophoresis (PAGE) (*8*) were performed on extracts of mouse red cells and of isolated parasites (*9*) and stained for SOD activity (*10*). The mobilities of host and parasite SOD's were identical on PAGE (Fig. 2A) and IEF (Fig. 2B) ($pI \cong 5.1$).

These results suggest that the malarial SOD might be entirely of host origin. Indeed, we have obtained direct evidence that this is the case. If the plasmodial SOD originates from the host cell, the characteristics of this enzyme should depend on the species of infected animal. To test this, we infected weanling rats

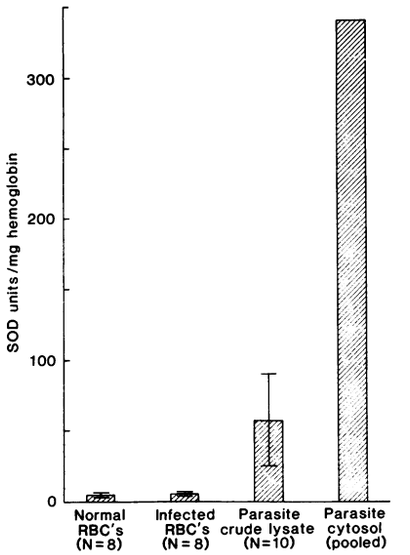

Fig. 1. Ratio of SOD activity to hemoglobin content of normal and *P. berghei*–infected murine red blood cells (RBC's), and of isolated *P. berghei* (*5*) (mean ± standard deviation). The parasite cytosol was prepared by ultracentrifugation (100,000*g*) of homogenized parasites (pooled samples from 12 mice) for 1 hour (*12*). Mean SOD activities per milligram of protein in these preparations (see Table 1) were 4.4 ± 0.5 (red cells), 6.1 ± 1.5 (infected red cells), 12.3 ± 4.1 (crude parasite lysates) and 16.8 (parasite cytosol). Note the much greater ratio of SOD to hemoglobin in parasite-associated material.

with the same strain of *P. berghei*. Examination by PAGE (Fig. 2A) and IEF (Fig. 2B) of parasite cell free extracts isolated from these animals revealed bands of activity identical to those from extracts of uninfected rat erythrocytes. The isoelectric point of the parasite-associated SOD ($pI \cong 5.7$) was characteristic of rat red cell SOD and quite distinct from that of parasites isolated from infected mice.

Thus several lines of evidence indicate that SOD activity associated with isolated *P. berghei* originates from the host cell. First, although the total cellular SOD activity declines within infected erythrocytes, substantial SOD is present within isolated *P. berghei*. In progressively more heavily infected animals, the host cytoplasm SOD activity decreases whereas that associated with isolated parasites increases reciprocally. Second, the SOD activity of isolated *P. berghei* is as sensitive to cyanide inhibition as is that of the host cell. In this regard, malarial SOD is unlike those reported for any other parasitic protozoan. Third, control studies (not shown) indicate no detectable passive adsorption of exogenously added (bovine) SOD by isolated *P. berghei*. Finally, the electrophoretic properties of *P. berghei*–associated SOD depend on whether the parasite is raised in mice or rats and, in both cases, the parasite SOD is electrophoretically indistinguishable from the host cell enzymes.

These observations indicate that *P. berghei* lacks an endogenous SOD and may depend on the host cell as a source of active enzyme. The absence of detectable SOD synthesized by the parasite itself is especially surprising in an organism living in such an oxygen-rich environment. The fact that it does not produce SOD is probably due to adoption of the active host cell enzyme. To our knowledge, this is the first example of direct parasitic incorporation of a host cell enzyme. The uptake of nutrients and cofactors by malarial parasites is well established (*11*). Malarial parasites rapidly endocytose and digest host cell hemoglobin (*11*) and, probably, other cytoplasmic constituents. SOD that is taken up along with the hemoglobin may es-

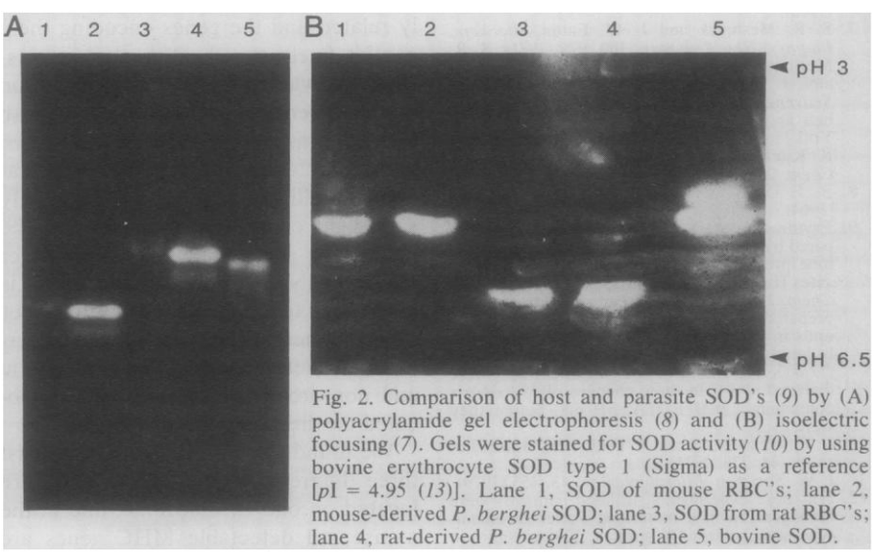

Fig. 2. Comparison of host and parasite SOD's (*9*) by (A) polyacrylamide gel electrophoresis (*8*) and (B) isoelectric focusing (*7*). Gels were stained for SOD activity (*10*) by using bovine erythrocyte SOD type 1 (Sigma) as a reference [$pI = 4.95$ (*13*)]. Lane 1, SOD of mouse RBC's; lane 2, mouse-derived *P. berghei* SOD; lane 3, SOD from rat RBC's; lane 4, rat-derived *P. berghei* SOD; lane 5, bovine SOD.

cape digestion and either remain within the food vacuole or emigrate into the parasite cytoplasm. These results serve to emphasize the complexity and intricacy of parasite dependence on host resources. Knowledge of such dependence may, in the future, suggest alternative approaches to the control of parasitic diseases.

ALEXANDRA S. FAIRFIELD
STEVEN R. MESHNICK
Departments of Microbiology and *Medicine, Cornell University Medical College, New York 10021*
JOHN W. EATON
Department of Laboratory Medicine and Pathology, University of Minnesota School of Medicine, Minneapolis 55455

References and Notes

1. I. Fridovich, *Annu. Rev. Biochem.* **44**, 147 (1975); J. M. McCord, B. B. Kaele, Jr., I. Fridovich, *Proc. Natl. Acad. Sci. U.S.A.* **68**, 1024 (1971).
2. R. P. Hebbel, J. W. Eaton, M. Balasingam, M. H. Steinberg, *J. Clin. Invest.* **70**, 1253 (1982).
3. N. L. Etkin and J. W. Eaton, in *Erythrocyte Structure and Metabolism*, G. J. Brewer, Ed. (Liss, New York, 1975), p. 219; M. J. Friedman, *Nature (London)* **280**, 245 (1979).
4. M. L. Salin and J. M. McCord, *J. Clin. Invest.* **54**, 1005 (1974).
5. *Plasmodium berghei* (NYU-2) was maintained in NCS or BALB/c mice by intraperitoneal injection of infected blood to yield a maximum parasitemia (≥ 75 percent) in 4 days with no increase in reticulocyte number. Mice were bled intracardially with heparin, and Hanks balanced salt solution (HBSS) was used to wash the red blood cells. White cells and platelets were removed by using a Sigmacell/Cellulose column [E. Beutler, *Red Cell Metabolism: A Manual of Biochemical Methods* (Grune & Stratton, New York, 1975), p. 10]. Erythrocytes were washed three times in HBSS after which parasites were isolated by saponin lysis [A. Zuckerman, *Bull. WHO* **37**, 431 (1967)]. Parasites were sedimented by centrifugation at 4500*g* for 5 minutes and washed four times in HBSS. The initial parasite-free supernatant was used to represent the host red blood cell cytoplasm. All fractions were frozen and thawed and Triton X-100 was added to 0.05 percent before the SOD, hemoglobin, and protein determinations were done. Hemoglobin was measured with Drabkin's reagent as described in N. W. Teitz *et al.*, *Fundamentals of Clinical Chemistry* (Saunders, Philadelphia, 1976), p. 412. Total protein was determined by the method of M. M. Bradford [*Anal. Biochem.* **72**, 248 (1976)].
6. U. Suthipark, J. Krungkrai, A. Jearnpipatkul, Y. Yuthavong, B. Panijpan, *J. Parasitol.* **68**, 337 (1982).
7. S. R. Meshnick and J. W. Eaton, *Biochem. Biophys. Res. Commun.* **102**, 970 (1981); S. R. Meshnick, N. L. Trang, K. Kitchener, A. Cerami, J. W. Eaton, in *Oxy Radicals and Their Scavenger Systems: Molecular Aspects*, G. Cohen and R. Greenwald, Eds. (Elsevier, New York, in press). N. L. Trang, S. R. Meshnick, K. Kitchener, J. W. Eaton, A. Cerami, *J. Biol. Chem.* **258**, 125 (1983).
8. B. J. Davis, *Ann. N.Y. Acad. Sci.* **121**, 404 (1964).
9. Erythrocyte SOD-containing extracts were prepared by chloroform-ethanol treatment and acetone precipitation of normal red blood cell lysates [J. M. McCord and I. Fridovich, *J. Biol. Chem.* **244**, 6049 (1969)]. Parasite extracts were prepared by sonication (three times for 20 seconds in 25 m*M* tris buffer, *p*H 6.8) of washed isolated parasites followed by centrifugation (27,000*g* for 1 hour at 4°C). Both extracts were dialyzed at 4°C in three changes of 25 m*M* tris containing 1 m*M* EDTA, *p*H 6.8, before they were used.
10. C. O. Beauchamp and I. Fridovich, *Anal. Biochem.* **44**, 276 (1971).
11. I. W. Sherman, *Microbiol. Rev.* **43**, 453 (1979).
12. For these experiments, isolated parasites were homogenized in a motorized Potter-Elvehjem-type homogenizer (40 strokes, 1200 rev/min) in sucrose-TKM buffer [I. W. Sherman and L. A. Jones, *J. Protozool.* **26**, 489 (1979)]. Homogenates were centrifuged at 100,000*g* for 1 hour and the supernatants were dialyzed and assayed for protein, hemoglobin, and SOD activity.
13. J. V. Bannister, W. H. Bannister, E. Wood, *Eur. J. Biochem.* **18**, 178 (1971).
14. We thank A. Cerami and J. Haldane for discussions and K. Kitchener, C. Schneider, and S. Swiecicki for technical assistance. Supported by NIH grants AI 16975 and HL 16833, by Rockefeller Foundation grant RF 81060, and by grants from the WHO/UNDP/World Bank Special Programme for Research and Training in Tropical Diseases. J.W.E. is a recipient of an NIH Research Career Development Award and S.R.M. is a recipient of an Irma T. Hirschl Trust Career Scientist Award.

4 April 1983; revised 13 June 1983

Multigene Family for Sarcomeric Myosin Heavy Chain in Mouse and Human DNA: Localization on a Single Chromosome

Abstract. *Cloned myosin heavy chain DNA probes from rat and human were hybridized to restriction endonuclease digests of genomic DNA from somatic cell hybrids and their parental cells. The mouse myosin heavy chain genes detectable by this assay were located on chromosome 11, and three different human sarcomeric myosin heavy chain genes were mapped to the short arm of chromosome 17. A synteny between myosin heavy chain and two unrelated markers, thymidine kinase and galactokinase, was found to be preserved in the rodent and human genomes.*

Myosin heavy chain (MHC) is a major protein of the contractile apparatus. There are many forms of MHC, and their expression is developmentally regulated and tissue-specific (*1*). Analyses of complementary DNA (cDNA) and genomic clones indicate that sarcomeric MHC is encoded by a conserved, multigene family consisting of at least 13 different members (*2–6*). It is important to determine whether the genes of such a multigene family are in tandem arrays, are clustered in an interspersed pattern on one or several chromosomes, or are individual genes at multiple loci. Mammalian globin genes exist in small clusters that are dispersed on two chromosomes (*7*), whereas *Drosophila* actin genes and chicken tubulin genes are located at multiple loci (*8, 9*). Two other multigene families, the histocompatibility genes and the major urinary protein genes of the mouse exist in large complexes on one chromosome each (*10–12*).

Because MHC proteins are functionally related and the genes encoding them exhibit finely tuned differential expression, knowledge about the organization of these genes may help to distinguish molecular aspects of their control. Also, knowledge about the genomic organization of multigene families will ultimately shed light on molecular evolution. The nucleotide sequence of the messenger RNA (mRNA) for sarcomeric MHC is not related to the sequence of mRNA for nonsarcomeric MHC (*2*). It will be interesting to determine whether sarcomeric and nonsarcomeric MHC genes are located on the same chromosome.

We found that, in the human, at least three different skeletal MHC genes are located on one chromosome, and in the mouse, all detectable MHC genes are located on one chromosome. Furthermore, a syntenic relation between MHC genes and two unrelated markers has been preserved in the mouse and human genomes. The chromosomal localization was determined by hybridization of cloned MHC DNA probes to restriction enzyme digests of genomic DNA from somatic cell hybrids and their parental cells. The human MHC genes were assigned to the short arm of chromosome 17 and the mouse MHC genes to chromosome 11.

In these chromosomal localization studies, two types of somatic cell hybrids were used: mouse × rat and human × mouse. Somatic cell hybrids formed between primary mouse cells and the thymidine kinase–deficient (TK^-) rat cell line FT-1 retain a complete set of rat chromosomes together with smaller numbers of mouse chromosomes. Likewise, cell hybrids between primary human cells and a permanent mouse cell line segregate human chromosomes, retaining the full mouse complement. Mouse × hamster and human × mouse hybrids and their use in making gene assignments have been described (*13–16*).

A cDNA clone of rat MHC was used as a hybridization probe in the assignment of the mouse MHC gene complex. This clone corresponds to embryonic skeletal MHC mRNA and represents 630 nucleotides at the 3′ end of the mRNA (*17*). Vertebrate sarcomeric MHC genes are highly conserved (*2*). Because of cross-hybridization, an enzyme that distinguished restriction fragment patterns of mouse and rat MHC genes was needed. Hind III resulted in the most polymorphism between these species (data not shown).