could expect a supplemental reduction of axons. Indeed, we found that an additional half million optic fibers or roughly 50 percent of the adult number are deleted during a second, slow phase of elimination that extends into postnatal life (Fig. 3). This late phase of axonal reduction may serve functions unrelated to the establishment of connections mediating binocular vision since cell death, withdrawal of axons, or both seem to be a ubiquitous feature of the developing brain (*17*).

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A Developmentally Regulated Neuraminidase Activity in *Trypanosoma cruzi*

Abstract. *The human pathogen* Trypanosoma cruzi *(Y strain) contains a neuraminidase activity that varies widely in the different developmental stages of the parasite. The specific neuraminidase activity of infective trypomastigotes obtained from tissue culture and from the bloodstream of infected mice is 7 to 15 times higher than that of the acellular culture forms. Amastigotes were devoid of enzyme activity. The enzyme has a* p*H optimum of 6.0 to 6.5. Live trypanosomes released sialic acid from human erythrocytes and plasma glycoproteins. Several sialyl compounds were hydrolyzed by the parasite, but the best substrate was the protein orosomucoid. Erythrocytes from infected mice with* T. cruzi *parasitemia were agglutinated by peanut lectin and the hemagglutination titer was correlated with the degree of parasitemia.*

Chagas' disease, which results from infection with the protozoan parasite *Trypanosoma cruzi*, is characterized by an acute illness that is followed in some patients by chronic cardiac and gastrointestinal sequelae (*1*). Little is known about the molecular basis of the pathogenic events.

In the experiments described here a developmentally regulated neuraminidase activity was discovered in *T. cruzi* that may be relevant to the pathogenesis of Chagas' disease. Viable parasites released sialic acid from human cells and serum glycoproteins in vitro and from erythrocytes of mice experimentally infected with *T. cruzi*. Erythrocytes that had been incubated with *T. cruzi* were agglutinated by peanut lectin (PNA).

It is widely accepted that sialic acid is involved in physiological processes of mammalian cells and glycoproteins (*2*). Since it is a relatively strong acid (pKa = 2.6), it is essentially completely deprotonated at physiological pH, thereby providing a negative charge at the surface of cells (*3*). Treatment of cells with neuraminidase alters this charge. Plasma glycoproteins and blood cells that have been desialylated are rapidly removed from the circulation and accumulate in the spleen and liver (*4*). Lymphocytes treated with neuraminidase show altered functional properties (*5*). Certain antigens present on the surface of intact cells are more extensively exposed after neuraminidase digestion (*6*). Neuraminidase also has a functional effect on cardiac cells (*7*) and on the neuromuscular junction (*8*). These cells are potentially affected in *T. cruzi*–infected mammals.

The developmental forms of *T. cruzi* were obtained as follows: Epimastigotes were from 5-day-old cultures in an ox liver infusion mixture containing tryptose and supplemented with 10 percent fetal calf serum at pH 7.2 (LIT) (*9*). Trypomastigotes were purified from 20- to 30-day-old LIT cultures on a DEAE-cellulose column (*9*). Bloodstream trypomastigotes were isolated from heparinized blood of 6-day infected CF-1 mice that had been irradiated 2 days before infection with 600 rad from a cesium source (*9*). Trypomastigotes were also obtained from supernatants of primary cultures of newborn human foreskin fibroblasts (*10*). Amastigotes were obtained from the fibroblast cultures 2 to 3 days after the trypomastigotes were harvested. They were washed twice with RPMI-1640 medium and isolated from the 20:25 percent interface of a discontinuous gradient of 15, 20, and 25 percent metrizamide after centrifugation at 1000g for 60 minutes. If the parasites were to be assayed live, they were washed three times in RPMI-1640 medium containing protease inhibitors [2 μM leupeptin, 2 mM phenylmethylsulfonyl fluoride (PMSF)] and 0.02 percent azide as preservative. In the assays with lysed trypanosomes, the organisms ($\sim 10^8$ per milliliter) were centrifuged at 1000g for 10 minutes. The pellet was resuspended to the initial concentration in ice-cold distilled water containing 2 μM leupeptin, 1 mM PMSF, and 0.02 percent sodium azide and washed twice in distilled water at 100,000g for 60 minutes before the enzyme assay (*11*).

The rate of hydrolysis of several sialyl compounds was linear for at least 3 hours in experiments with live parasites or with suspensions of trypanosomes lysed in distilled water under the assay conditions (*11*). The relation between trypanosome concentration (viable and lysed organisms) and enzyme activity

was also linear. Curves for *p*H plotted against activity revealed *p*H optima of 6.0 to 6.5 for both the epimastigote and trypomastigote neuraminidase, regardless of whether the cells were live or previously lysed in water.

The specific neuraminidase activity of *T. cruzi* varied greatly among the developmental stages of the parasite. The enzyme was present to about the same extent in epimastigotes and trypomastigotes isolated from LIT cultures (Table 1). The specific neuraminidase activity of the trypomastigotes obtained from cells in tissue culture was 7 to 15 and 2 to 3 times higher than that of the corresponding trypomastigotes from LIT cultures and the blood of mice, respectively (Table 1). In contrast, amastigotes were devoid of detectable enzyme activity.

The rate of hydrolysis of six different substrates by the neuraminidase of the developmental forms of *T. cruzi* is shown in Table 1. The protein orosomucoid (α-1 acid glycoprotein) was hydrolyzed at a higher rate than all other substrates. Colominic acid, ovomucoid, and bovine submaxillary mucin were comparatively poor substrates. The trypomastigotes from fibroblast cultures and from the blood of mice were the most active of the developmental stages of *T. cruzi*, whereas epimastigotes and trypomastigotes isolated from LIT cultures had relatively low enzyme activity.

The *T. cruzi* neuraminidase was active against human cells. Incubation of live, freshly harvested trypomastigotes from fibroblast cultures with human A, B, or O erythrocytes (Fig. 1) released sialic acid in a time-dependent fashion. That

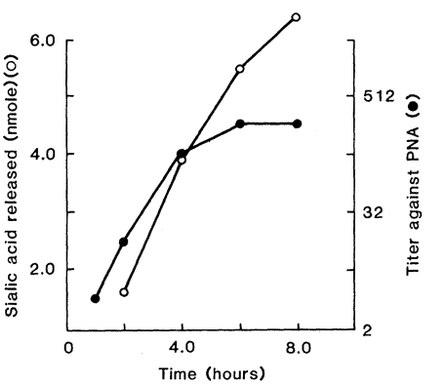

Fig. 1. Release of sialic acid from human erythrocytes (O Rh^+) by live trypomastigotes (Y strain). Erythrocytes (4×10^9 cells) and fibroblast trypomastigotes (4×10^7 cells) were incubated at 37°C in PBS, *p*H 6.5, for the times indicated. Free sialic acid was determined by the thiobarbituric acid method after purification through an anion exchange column (*17*). Washed erythrocytes were titrated against peanut lectin (initial concentration, 1.1 mg/ml) in microtiter plates (*12*).

Table 1. Hydrolysis of various substrates by the neuraminidase of *T. cruzi* (Y). Neuraminidase activity was measured with equivalent amounts of each substrate (100 nmole of bound sialic acid) (*12*). The results represent the average of two experiments. The parasites were lysed in ice-cold distilled water before assay of the enzyme at *p*H 6.5. The bloodstream trypomastigotes were obtained from γ-irradiated mice. N.D., not done.

Substrate	Specific activity per hour (nanomoles of sialic acid per 10^8 trypanosomes)			
	Epimastigotes	Trypomastigotes		
		LIT	Fibroblast	Bloodstream
α-1 Acid glycoprotein	2.6	2.1	39.0	15.0
Fetuin	1.3	N.D.	16.0	N.D.
Sialyl lactose	1.5	1.3	16.0	8.0
Colominic acid	0.3	N.D.	2.5	N.D.
Ovomucoid	0.2	N.D.	1.5	N.D.
Bovine submaxillary mucin	0.2	N.D.	1.4	N.D.

the free sialic acid indeed originated from the erythrocytes was reflected in the hemagglutination results with PNA. Peanut lectin does not normally agglutinate mature, fully differentiated cells unless the cells are first treated with neuraminidase to expose receptor sites (mainly DGalβ1 → 3DGalNAc) (*12*). As shown in Fig. 1, the agglutinating activity of PNA for human O erythrocytes became apparent only after incubation of the trypanosomes with the red cells, the increase in the hemagglutination titer being concomitant with the rise in free sialic acid in the reaction mixtures.

Since neuraminidase activity was found in bloodstream trypomastigotes harvested from γ-irradiated mice, and because of the implications for the pathogenesis of Chagas' disease, the activity of neuraminidase was determined in *T. cruzi*–infected mice. Mice were inoculated with *T. cruzi* and the erythrocytes of the infected animals were analyzed for agglutination with PNA. As shown in Fig. 2, parasites began to appear in the bloodstream 4 days after inoculation and reached a maximum concentration 3 to 4 days later. Concomitantly, the erythrocytes of such infected mice became agglutinable by PNA, the hemagglutination titer being somewhat correlated with the degree of parasitemia. The erythrocytes from uninfected mice and from the infected mice before they developed parasitemia were not agglutinated by the lectin. Further evidence for the desialylation of the erythrocytes by the bloodstream trypomastigotes was obtained by testing the activity of the parasites toward human erythrocytes in vitro. Trypomastigotes (1×10^6) were isolated from mice 8 days after infection and incubated with human O erythrocytes (5×10^7) for 3 hours at 37°C, in 0.5 ml of phosphate-buffered saline (PBS), *p*H 7.2. The cells were then washed twice with PBS and tested with PNA. The *T. cruzi*–treated erythrocytes were agglutinated by PNA at a minimum concentration of 125 μg of lectin per milliliter, whereas the erythrocytes incubated in the absence of *T. cruzi* were not agglutinated.

The finding of neuraminidase activity in *T. cruzi* may be relevant to the pathogenesis of Chagas' disease. In view of the desialylation of erythrocytes in vitro and in vivo by live trypanosomes, and in view of the crucial role (*4*) played by surface-bound sialic acid in the normal life-span of erythrocytes, platelets, and lymphocytes, the intense thrombocytopenia, anemia, and leukopenia present in *T. cruzi*–infected mice (*13*) could be due to the *T. cruzi* neuraminidase. In this

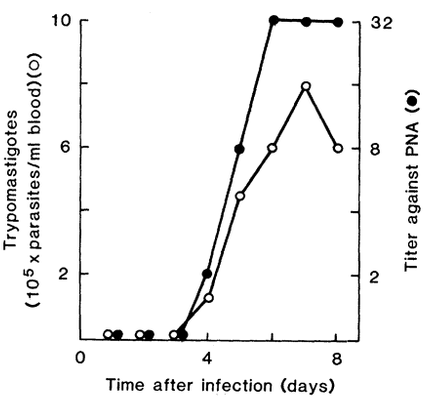

Fig. 2. Neuraminidase activity in mice infected with *T. cruzi*. CF-1 mice ($N = 5$) were injected intraperitoneally with 7×10^4 bloodstream trypomastigotes (Y strain). Blood was obtained from a tail vein at daily intervals and the parasites were counted in a hemocytometer. The erythrocytes were assayed for agglutination with PNA (initial concentration, 1.1 mg/ml) in 0.01*M* sodium phosphate buffered saline containing bovine serum albumin (2 mg/ml) and 30 percent ethylene glycol to increase the sensitivity of the assay. At the end of 8 days, the mice were killed and the blood was pooled. The parasites were partially separated from the blood cells in a Metrizamide gradient (*9*) and assayed for neuraminidase activity in vitro with human erythrocytes as described in Fig. 1. The results are shown as the average of two experiments.

regard, a neuraminidase activity has been detected in African trypanosomes, and the anemia of cattle experimentally infected with *T. vivax* has been related to this enzyme activity (*14*). Moreover, it is possible that the enzyme plays a role in adsorption to and penetration of the trypanosome into host cells and in the release of parasites from infected cells.

It is of interest that the highest level of neuraminidase activity was found in trypomastigotes (Table 1), the trypanosome form that infects host cells and is released from them into the circulation to spread the infection. A role for neuraminidase in infection was first described with the mixoviruses (*15*), and a correlation has been found between the protection of individuals vaccinated with influenza virus and the titer of antibodies to neuraminidase (*16*). It would therefore be interesting to determine whether patients with Chagas' disease, or experimental animals infected with *T. cruzi*, develop antibodies to neuraminidase and whether such antibodies have functional significance in the trypanosomal infection. The applicability of this system to other parasitic infections, such as malaria, should also be investigated.

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11. For the neuraminidase assay, 50 μl of a suspension of trypanosomes (1×10^6 bloodstream or tissue culture trypomastigotes and 1×10^7 epimastigotes or amastigotes) was incubated at 37°C with 20 μl of 1*M* sodium phosphate buffer (*p*H value indicated in each experiment), 50 μl of an aqueous solution of substrate containing 100 nmole of bound sialic acid, and 80 μl of distilled water. At the end of the incubation period, 20 to 50 μl of reaction mixture was centrifuged in a Beckman microfuge and the supernatant was analyzed for free sialic acid by the periodate–thiobarbituric acid method [D. Aminoff, *Biochem. J.* **81**, 384 (1961)]. Enzyme and substrate controls were incubated concurrently and the corresponding readings subtracted from that obtained with the complete enzyme system. One unit of neuraminidase activity is defined as the amount of enzyme that releases 1 nmole of sialic acid per hour under the conditions of the assay. Specific activities are expressed as units of neuraminidase activity per 10^8 trypanosomes.
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Biological Control of Yellow Nutsedge with the Indigenous Rust Fungus *Puccinia canaliculata*

Abstract. *Yellow nutsedge* (Cyperus esculentus *L.) is a serious weed problem in the United States and other countries. An indigenous rust fungus* [Puccinia canaliculata *(Schw.) Lagerh.], pathogenic on yellow nutsedge, was released in early spring as a potential biological control agent. The fungus inhibited nutsedge flowering and new tuber formation. The fungus also dehydrated and killed nutsedge plants. The successful control of yellow nutsedge by a rust epiphytotic under experimental conditions demonstrates the potential use of the rust in an integrated weed management system.*

Yellow nutsedge (*Cyperus esculentus* L.) is thought to be one of the world's most troublesome weeds (*1*). Hauser (*2*) described nutsedge as a worldwide plague. This weed has been a serious pest for many decades. In recent years it has spread rapidly throughout the United States and is now the most troublesome perennial weed in most of the Midwest (*3*).

Yellow nutsedge reproduces prolifically. One tuber planted in a field in Minnesota produced 1900 plants and 6900 tubers in 1 year (*4*). In Georgia 622 tubers were produced from one tuber in 17 weeks (*5*).

In spite of considerable effort, biocontrol methods for yellow nutsedge are not yet effective for field use (*3*). Rust caused by *Puccinia canaliculata* (Schw.) Lagerh. was first described in 1832. In 1906, Arthur demonstrated that *Xanthium* sp. was an alternate host, an indication that the pathogen is a macrocyclic heteroecious rust (*6*). Another possible alternate host is giant ragweed, *Ambrosia trifida* L. To our knowledge, no research has been reported on this rust except by us (*7, 8*). Researchers have observed the rust on yellow nutsedge in many locations in the United States and Canada. However, it usually does not appear until August and does not increase substantially until September. By then, the nutsedge has produced new seed and tubers and its life cycle is unaffected by the rust.

The objective of our research program is to develop an integrated weed management system (IWMS) for yellow nutsedge by integrating biological, chemical, and cultural practices. Selective and nonselective chemical treatments are available for the control of yellow nutsedge (*4*), and cultural and chemical practices can be used to reduce this pest without reducing the yield of soybeans and corn (*3*). However, acceptable control of yellow nutsedge is difficult to achieve in horticultural crops. We present here data on the effect of this rust fungus on yellow nutsedge in southern Georgia from 1978 to 1981.

An epiphytotic of rust was observed on a dense stand of yellow nutsedge in September 1978 on a research farm near Tifton, Georgia, and in August–September 1979 a severe rust epiphytotic developed on this nutsedge. In 1980, we carried out weekly observations and found a few plants with rust in June. The infected nutsedge was located in vegetable re-

Table 1. Effect of rust fungus on nutsedge 60 days after rust release. Each mean is the average of four replications. In all cases listed, the means in each column were different at the 5 percent level (*t*-test).

Nutsedge sample	Leaf area diseased (%)	Root growth (%)	Fresh weight per plant (g)	Dry weight per plant (g)	Dry matter (%)
Rust-infected	78.5	18.5	5.9	1.8	29.8
Control*	10.5	87.0	13.7	2.9	21.1

*Weekly application of chlorothalonil.