(*8*) (Table 1). For the birds the average difference is 5 per mil, and for the marine mammals, 3 per mil. A ^{15}N enrichment of this magnitude is consistent with the observation that the $\delta^{15}N$ values of an animal's tissues are about 3 per mil more positive than that of its diet (*7*). In some cases, this trophic-level effect will have to be considered when human bone collagen $\delta^{15}N$ values are used for dietary reconstruction.

We have shown that the $\delta^{15}N$ values of bone collagen can be used to estimate the marine and terrestrial components of diets among historic and prehistoric human populations. In some cases, determination of both the $\delta^{15}N$ values and the $\delta^{15}C$ values of bone collagen will produce a more reliable reconstruction of this aspect of diet than analysis of only one isotope ratio. For example, $\delta^{13}C$ values of bone collagen of humans whose diet consisted of equal amounts of C_4 plants, C_3 plants, and terrestrial mammals would mimic those resulting from a completely marine diet (*4–7, 22*), while $\delta^{15}N$ values from humans subsisting on large amounts of tropical reef organisms, such as the Bahamian group, do not reflect the marine origins of their diet. Use of both the $\delta^{13}C$ and $\delta^{15}N$ values of bone collagen to estimate the composition of the diet would clarify each of these situations.

MARGARET J. SCHOENINGER*
MICHAEL J. DENIRO†
Department of Earth and Space Sciences, University of California, Los Angeles 90024
HENRIK TAUBER
Carbon-14 Dating Laboratory, National Museum, Ny Vestergade 10, Copenhagen K, Denmark

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$$\delta^{13}C = \left[\frac{(^{13}C/^{12}C)_{sample}}{(^{13}C/^{12}C)_{standard}} - 1\right] \times 1000 \text{ per mil}$$

$$\delta^{15}N = \left[\frac{(^{15}N/^{14}N)_{sample}}{(^{15}N/^{14}N)_{standard}} - 1\right] \times 1000 \text{ per mil}$$

The standards are the Pee Dee belemnite (PDB) carbonate for $\delta^{13}C$ values and atmospheric nitrogen (AIR) for $\delta^{15}N$ values.
20. Nitrogen fixation in coral reefs occurs at the rate of 25 g/m^2 per year, whereas in the shallow waters of the open ocean it occurs at 0.1 g/m^2 per year. Nitrogen fixation is also high in seagrass meadows, salt marshes, and mangrove swamps [D. G. Capone and E. J. Carpenter, *Science* **217**, 1140 (1982)].
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* Permanent address: Department of Cell Biology and Anatomy, Johns Hopkins University School of Medicine, Baltimore, Md. 21205.
† M.J.D. is also in the Archaeology Program.
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Atypical Pulmonary Thrombosis Caused by a Toxic Cyanobacterial Peptide

Abstract. *Parenteral injection into mice of a toxic pentapeptide isolated from the cyanobacterium* Microcystis aeruginosa *induced thrombocytopenia, pulmonary thrombi, and hepatic congestion. The lethality of the toxin was unaffected by several anticoagulants. The acute liver damage that follows injection of the toxin has been attributed to direct action on liver cells but may be due to hypoxemia, heart failure, and shock.*

The acute toxicity to mammals of the cosmopolitan freshwater cyanobacterium *Microcystis aeruginosa* (*1–3*) is attributed to a pentapeptide (*4–6*). A leucine (L)- and arginine (R)-containing pentapeptide (toxin-LR) occurs frequently in toxic strains of *M. aeruginosa* (*7, 8*). Extracts of *M. aeruginosa* parenterally injected into rodents elicit hepatotoxic effects, including sinusoidal congestion, hemorrhage, and necrosis (*9–12*). A lethal dose of purified toxin-LR induces multiple thrombi in the lung as well as hepatic changes. Liver toxicity observed on gross examination and by light microscopy may be the result of hypoxemia, heart failure, and shock, as expected from acute pulmonary vascular occlusion.

Studies were performed with 8- to 12-week-old female Swiss albino mice of the Hale-Stoner strain (*13*). The median lethal dose (LD_{50}) of toxin-LR was about 0.06 μg per gram of body weight. The route of injection, whether intravenous or intraperitoneal, did not substantially affect toxicity. Mice did not react to toxin-LR until 20 to 40 minutes after injection. Hunched posture, immobility, and piloerection were then observed during increasingly frequent and longer time intervals. This reduced activity was interrupted by apparently unprovoked leaps. Such behavior was followed by lassitude, continued piloerection, tachypnea, and subcostal retraction. The ears and digits of affected mice became pale but not cyanotic. Mice surviving the injections by more than 2 hours usually lost all signs of toxicity within a few hours thereafter. If death ensued, it was preceded by pallor of eyes and tail, syncope, and then coma associated with occasional respiratory gasps. The usual time range between the injection of marginally lethal doses (~ LD_{50}) of toxin and death was not noticeably altered when substantially supralethal doses (~ 4 times LD_{50}) were given.

Necropsies were performed immediately after the mice were killed by ether inhalation. Histological sections (5 μm) of formalin-fixed vital organs were stained with hematoxylin and eosin. Adjacent lung sections were stained by a fast phosphotungstic acid–hematoxylin method (PTAH) (*14*).

Livers appeared dark red and markedly enlarged (~ 50 percent increase in fresh weight) within 2 hours of injection with a lethal dose of toxin-LR. A thin film of pink ascitic fluid was sometimes noted. The cerebral cortex was slightly swollen and pale. No other abnormalities were observed on gross examination of the unfixed vital organs of mice given

lethal injections of the toxin. Histologic examination showed marked, predominantly centrilobular, congestion of sinusoids and focal necrosis of the liver, frequently associated with interstitial extravasation of blood. Thrombi were not observed in the liver.

Mice, moribund or dead within 2 hours of injection by toxin-LR, had multiple pulmonary thrombi. Such thrombi were first observed by Falconer *et al.* (*12*) in mice given injections of a closely related toxic peptide of *M. aeruginosa*. Pulmonary thrombi induced by toxin-LR were not attached to vascular endothelium, and adjacent endothelial cells were not swollen. The thrombi comprised irregular clusters (< 15 μm in diameter) of granules. Individual granules were about 1 μm in diameter. The thrombi were chromophobic in sections stained with hematoxylin and eosin, but were readily identified by their deep purple color in PTAH sections. Light microscopy revealed neither fibrin strands nor trapped erythrocytes in the thrombi.

The association of pulmonary thrombi with toxin lethality was tested. One randomly selected section of lung (5 μm, stained with PTAH) from each of nine mice given a lethal or sublethal dose of toxin and a section from each of four mice that had not been given the toxin were coded and then examined by a pathologist who had no knowledge of their identities. Each of the 13 sections was scored for the presence of at least one purple-stained intravascular thrombus. The presence of thrombi in these randomly selected, PTAH-stained sections of lung was exclusively associated with parenteral injection of a rapidly lethal dose of toxin (Table 1).

Livers from toxin-injected mice showed severe congestion whether or not the mouse survived the initial critical 2-hour postinjection period. Occasionally, a mouse injected with a lethal dose of toxin-LR survived the critical 2 hours but did not recover completely, remaining listless until it died several hours or several days later. Focal fatty degeneration of the liver and active regeneration of liver cells were seen several days after sublethal doses of toxin-LR. The reasons for delayed deaths of mice injected with toxin-LR are unknown.

Mean values for coagulation tests (*15–17*) in untreated mice were: whole blood clotting time, 3.5 minutes; euglobulin lysis time, 5.5 hours; prothrombin time, 13 seconds; activated partial thromboplastin time, 2 minutes; fibrinogen, 350 mg/dl. No significant changes in these results occurred after injection of toxin-LR. However, starting one-half hour after injection, about 80 percent of blood platelets were rapidly removed from the circulation (Table 2).

Effects of anticoagulant prophylaxis on peptide toxicity were assessed. Heparin, acetylsalicylic acid, Malayan pit viper venom (*18*), streptokinase (*19*), and warfarin were tested (*20*). Mice were challenged by toxin-LR, 0.1 μg per gram of body weight given intraperitoneally, a dose that was at least 90 percent lethal within 2 hours. Ten mice were tested with toxin-LR at each dose level of an anticoagulant. Before each experiment, ten normal mice were challenged by toxin to confirm toxin potency. The lethality of the toxin was not affected by streptokinase at doses that shortened the euglobulin lysis time to about one-half normal, snake venom at doses that produced sustained incoagulability of blood in vitro, or by heparin, warfarin, or acetylsalicylic acid in doses up to 10 percent of the LD_{50}.

Toxin-LR is a potent pulmonary thrombogenic agent in mice. Three aspects of its thrombogenesis are atypical. First, it is unaffected by a variety of anticoagulants. Second, the thrombi comprise large and small clusters of granules without the fibrin and trapped erythrocytes that are seen in red thrombi. Since toxin-LR causes thrombocytopenia, it is suggested that these granular thrombi are platelet aggregates. Third, although a platelet-aggregating mixture of collagen and epinephrine (*21*) kills Hale-Stoner mice within 2 minutes of intravenous injection, ibuprofen, a cyclooxygenase and thromboxane synthesis inhibitor (*22*), appears to lessen the lethality of this mixture in a dose (50 μg/g) that offers no protection to toxin-LR-injected mice. An unknown se-

Table 1. Correlation between lethality of toxin-LR from *Microcystis aeruginosa* and pulmonary thrombosis in mice.

Mouse	Dose of toxin-LR (μg/g)	Route	Pulmonary thrombosis	Effect of toxin
1	Untreated		−	
2	0.20	Intravenous	+	Moribund when killed 60 minutes after toxin
3	Untreated		−	
4	Untreated		−	
5	0.20	Intravenous	+	Moribund when killed 70 minutes after toxin
6	0.10	Intravenous	+	Moribund when killed 80 minutes after toxin
7	0.05	Intravenous	−	Recovered from acute toxicity. Killed 22 hours after toxin
8	0.07	Intravenous	−	Recovered from acute toxicity. Killed 3 days after toxin
9	0.10	Intravenous	+	Moribund when killed 117 minutes after toxin
10	0.10	Intraperitoneal	+	Moribund when killed 76 minutes after toxin
11	0.10	Intraperitoneal	+	Moribund when killed ~ 90 minutes after toxin
12	0.20	Intraperitoneal	+	Moribund when killed 60 minutes after toxin
13	Untreated		−	

Table 2. Blood platelet counts and liver weights of mice after injection of toxin-LR. Blood platelet counts and liver weights are median values, with the range given in parentheses.

Minutes after injection	Mice (No.)	Liver weight (percent of body weight)	Blood platelet count ($\times 10^6$/mm^3)	Spearman's coefficient of rank correlation	Probability of rank correlation (percent)
Control*	10	6.5 (5.7–7.3)	1.5 (1.3–1.8)	+0.21	< 50
15	10	6.2 (5.6–6.5)	1.4 (0.9–2.1)	−0.30	< 50
30	10	7.1 (5.8–9.2)	1.3 (0.2–1.7)	+0.60	93
45	10	9.9 (7.5–10.7)	0.3 (0.1–1.1)	+0.14	< 50

*Normal mice; no toxin given.

quence of events leading to platelet aggregation could account for the half-hour delay in onset of thrombocytopenia following injection of toxin-LR.

Toxin-LR elicits hepatomegaly due to pooling of blood in and around liver sinusoids. The sharp rise in Spearman's rank correlation between platelet count and liver weight that occurred 30 minutes after injection (Table 2), when changes in these parameters were observed in some animals, indicates that thrombocytopenia and hepatomegaly were almost concurrent. We postulate that the hepatomegaly was due to pulmonary vascular occlusion, possibly by platelet thrombi, with secondary hypoxemia, heart failure, and shock.

D. N. SLATKIN
R. D. STONER
W. H. ADAMS

Medical Department,
Brookhaven National Laboratory,
Upton, New York 11973

J. H. KYCIA
H. W. SIEGELMAN

Biology Department,
Brookhaven National Laboratory

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Cell Polarity: Endogenous Ion Currents Precede and Predict Branching in the Water Mold *Achyla*

Abstract. *The hyphae of the water mold* Achyla bisexualis *generate electrical currents that enter the growing tips and leave farther back. An inward-moving current also precedes branching and predicts the site of branch emergence; during the branching process, the current at the original tip declines or even reverses transiently without any change in growth rate. The inward current probably acts as an early signal during branch differentiation. The flow of specific ions rather than the flow of electrical charge probably serves to localize growth.*

Fungi exemplify to an extreme degree the eukaryotic habit of polarized growth. The mechanisms by which cell polarity is established and maintained are not well understood. Much interest therefore was generated by the discovery of Jaffe and his co-workers that many growing and developing systems drive electrical currents through themselves. In two instances, the developing zygote of the brown alga *Pelvetia* and the germinating lily pollen grain, the site of current entry predicts the site of future outgrowth (*1*). We report here that growing hyphae of the water mold *Achyla bisexualis* also generate endogenous ion currents that appear to play an important role in the genesis and maintenance of polarized growth.

Transcellular ion currents were measured with a vibrating probe constructed as described by Jaffe and Nuccitelli (*2*). The sensing element of this instrument consists of a microelectrode tipped with a ball of platinum black (approximately 20 μm in diameter). The electrode vibrates over an amplitude of 30 μm and measures the small potential differences between the extremes of its sweep (as little as 20 nV). The potential differences are converted to current densities by use of Ohm's law and the known resistivity of the medium. The probe measures only the net flow of electrical current, which is a summation of the fluxes of individual ionic species; by convention, current refers to the flow of positive charge.

Figure 1 shows the current densities measured along the length of a hypha of *A. bisexualis* growing in DMA medium (*3*). The probe was vibrating perpendicular to the hyphal axis, and the ordinate therefore gives the magnitude of the current vector entering or leaving the membrane. The inward-flowing current extended 350 μm behind the tip, beyond which the current turned outward (*4*).

Hyphae growing in this medium branched frequently, converting a nongrowing region of the hyphal trunk into a new tip that elongated at the same rate as the original tip (5 μm/min). Branches never emerged closer than 150 μm to the old tip but appeared at random farther back. Branching had a marked effect on the current pattern, especially if the branch emerged more than 300 μm behind the tip. In the hyphae that we studied every new branch emerged from a region of inward current, and in nearly every case (25 out of 27) the branch was associated with a peak of inward current. Remarkably, current entry into the site of the future branch was routinely detected 20 minutes or more before there was any visible sign of branch emergence, and the intensity of inward current was maximal before the new branch could be observed. Figure 2 shows an example of this phenomenon; the arrows mark the positions at which branches were seen 20 minutes after this current pattern was recorded.

What effect does the emergence of a branch have on current flow into the original tip? Current into the old tip