sediment. It follows from Eqs. 2 and 3 that C and C_p may be expressed in terms of C_T under conditions of sorptive equilibrium in which the concentration of suspended solids, M, is known

$$C = \frac{C_T}{1 + K_p M} \quad (4)$$

$$C_p = \frac{C_T K_p M}{1 + K_p M} \quad (5)$$

Substituting these expressions for C_p and C into Eq. 1 gives the mass balance differential equation

$$\frac{dC_T}{dt} = \frac{W(t)}{V} - \frac{C_T}{t_o} - \frac{\Sigma k + k_1}{1 + K_p M} C_T - \frac{k_s K_p M}{1 + K_p M} C_T \quad (6)$$

Bioaccumulation follows the linear kinetics of uptake minus depuration

$$\frac{dF}{dt} = k_1 f_1 C_T / B - k_2 F \quad (7)$$

where $f_1 = C/C_T = 1/(1 + K_p M)$ is the fraction of dissolved dieldrin; k_2 is the depuration rate constant, estimated as $k_1/B(\mathrm{BCF})$; F is the whole-body fish residue level; and B is the fish biomass concentration (wet weight).

Equations 6 and 7 may be solved analytically for constant coefficients and simple pesticide loading functions, $W(t)$, or they may be integrated numerically. In the case of a pesticide ban, $W(t)$ might typically decline exponentially because of degradation by soil organisms. For a loading function declining exponentially at rate ω, the analytical solutions to Eqs. 6 and 7 are

$$C_T = C_{T_o} e^{-\delta t} + \frac{C_{T,\mathrm{in}_0}}{\varepsilon}(e^{-\omega t} - e^{-\delta t}) \quad (8)$$

$$F = F_o e^{-k_2 t} + \frac{k_1 f_1}{B} \times \left(\frac{C_{T_0}}{\gamma} e^{-\delta t} + \frac{C_{T,\mathrm{in}_0}}{\varepsilon\theta} e^{-\omega t} - \frac{C_{T,\mathrm{in}_0}}{\varepsilon\gamma} e^{-\delta t}\right) - \frac{k_1 f_1}{B} e^{-k_2 t}\left(\frac{C_{T_0}}{\gamma} + \frac{C_{T,\mathrm{in}_0}}{\varepsilon\theta} - \frac{C_{T,\mathrm{in}_0}}{\varepsilon\gamma}\right) \quad (9)$$

where $f_2 = C_p/C_T = K_p M/(1 + K_p M)$ is the fraction of particulate pesticide, C_{T_0} is the initial pesticide concentration in the lake, C_{T,in_0} is the initial total pesticide inflow concentration, ω is the rate of the exponentially declining inflow centration, $\alpha = (\Sigma k) f_1 + k_1 f_1 + k_s f_2$, $\gamma = k_2 - \alpha - (1/t_0)$, $\delta = \alpha + (1/t_0)$, $\varepsilon = \alpha t_0 + 1 - \omega t_0$, and $\theta = k_2 - \omega$.

Equations 6 and 7 are written for only one water compartment with sedimentation of suspended solids into the sediment compartment. It is straightforward to extend the analysis to a number of compartments with flow and bulk dispersive transport between compartments (*10*). The equations are linear and may be solved analytically or numerically.

Model coefficients and results are presented in Fig. 2. From the analysis it was determined that ~ 40 percent of the dieldrin that enters the reservoir is lost to the bottom by sedimentation and 50 percent is released through the dam gates of the reservoir, which has a short detention time. Uptake by fish accounts for ~ 10 percent of the dieldrin input due to the extremely large biomass of biota. The partitioning of dieldrin in the water column is 74 percent in the fish, 25 percent dissolved in the water, and less than 1 percent adsorbed by suspended solids. Mean residues in the edible tissue of bottom-feeding fish have declined below the FDA guideline value of 300 ppb. Photodegradation, volatilization, and biological degradation rates are negligible in the water column compared to transport rates, but an overall dieldrin degradation rate of 0.1 percent per day in sediment contributes to the gradual decline of sediment concentrations. Concentrations in fish and sediment are essentially in equilibrium with mean concentrations of dissolved dieldrin. Bottom-feeding fish accumulate dieldrin in proportion to their oil content (petroleum ether extraction). Therefore averages or composites for very oily fish tended to be higher than model predictions.

The prospect for a continued decline in dieldrin residues is good. Model projections indicate that by 1986, the residues in the flesh of bottom-feeding fish should average less than 100 μg/kg. Research is continuing on the role of sediment resuspension and food items on the bioaccumulation potential of the fishery.

JERALD L. SCHNOOR

Energy Division, Civil and Environmental Engineering, University of Iowa, Iowa City 52242

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Protein Antigens from *Staphylococcus aureus* Strains Associated with Toxic-Shock Syndrome

Abstract. Staphylococcus aureus *isolates from patients with toxic-shock syndrome have two antigenic proteins that distinguish these strains from isolates obtained from patients who do not have this syndrome and who are colonized or infected with* S. aureus. *These proteins may either represent antigenic markers, the toxin (or toxins), or toxic subunits that cause the clinical manifestations of this disease.*

The toxic-shock syndrome (TSS) is a recently recognized illness that primarily affects previously healthy young women (*1, 2*). The syndrome most often occurs during the menstrual period and is characterized by acute onset of high fever, vomiting, diarrhea, an erythematous rash with subsequent desquamation, and severe hypotension. The syndrome occurs with a frequency of approximately three in 100,000 women and has been associated with a 10 percent mortality (*3*). Clinical and epidemiologic studies of TSS have implicated vaginal colonization with *Staphylococcus aureus* and the use of tampons as risk factors for development of disease (*3*).

Although *S. aureus* has been implicated in TSS, this microorganism colonizes a significant proportion of the general population, and, in one study, vaginal colonization occurred in 7 percent of menstruating women (*3*). Thus far no useful distinction has been reported between *S. aureus* isolates from patients with TSS and those from either the nor-

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mal population or patients having more common staphylococcal diseases. Isolates from patients with TSS have been almost uniformly resistant to penicillin, but such resistance is quite common among *S. aureus*. Moreover, no current evidence suggests that isolates from TSS patients have a characteristic bacteriophage typing pattern or belong to a particular staphylococcal phage group. The clinical features of TSS, particularly in the absence of bacteremia, suggest the elaboration of a toxin as the underlying mechanism of illness. Although several toxins including exotoxin A (*4*) and an exfoliatin-like toxin (*1*) have been demonstrated in TSS isolates, no controlled study has shown a difference in the production of these toxins among *S. aureus* isolates from patients with and without TSS. Hence the finding of a distinguishing feature (or features) of *S. aureus* isolates from patients with TSS would help establish an etiologic role for *S. aureus*, furnish a useful epidemiologic tool, and perhaps clarify the pathogenesis of the clinical manifestations of TSS.

Previous epidemiologic studies by Lovett *et al.* have shown the utility of comparing patterns of immunologically reactive microbial proteins detected by antibodies present in the serums of patients (*5*). This method depends on the transfer of protein from a sodium dodecyl sulfate (SDS)-polyacrylamide gel to a solid matrix (*6*) and the identification of antigenic proteins by specific antiserums and iodinated staphylococcal protein A (*7, 8*).

We have used this technique to examine *S. aureus* isolates from patients with and without TSS and report that *S. aureus* isolates from these cases can be distinguished from controls. We prepared whole-cell proteins from 18-hour cultures of *S. aureus* by sonication and boiling. These proteins were separated by electrophoresis on an SDS-polyacrylamide gel and then transferred to nitrocellulose paper (NCP) by electrophoresis. The NCP was sequentially soaked in: (i) 1 percent bovine serum albumin to prevent nonspecific binding of antibody to NCP; (ii) antiserum from patients convalescing from TSS to form antigen-antibody complexes; and (iii) staphylococcal protein A labeled with ^{125}I which serves to identify antigenic protein by binding to the Fc protein of any bound immunoglobulin G (IgG) and subsequently producing identifiable bands after exposure to x-ray film.

We initially examined six isolates of *S. aureus* from patients with TSS. These isolates contained two proteins—protein 1 and protein 2 (molecular weights 30,000 and 33,000, respectively)—which were detected by ^{125}I-labeled protein A and convalescent serum from a patient who had TSS. These proteins were not present in laboratory strains of *S. aureus*. Variations in the preparation of cellular protein, that is, use of French pressure cells, sonication, boiling, and the addition of β-mercaptoethanol, did not alter the ability to detect the unique antigens.

On the basis of these data, we examined 52 isolates of *S. aureus* provided by the Center for Disease Control. These isolates were obtained from 32 patients with TSS (cases) and 20 patients colonized or infected with *S. aureus* but without symptoms of TSS (controls). The isolates were matched insofar as possible with respect to the site of isolation, sex, and other epidemiologic factors. The strains were known to us only by an identifying number, and the distribution of the case and control strains was not decoded until our analysis was completed.

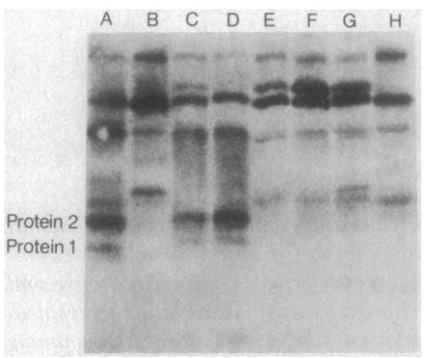

Fig. 1. Autoradiograph of whole-cell protein preparations of *S. aureus* isolates from cases of TSS (A, C, and D) and control isolates from individuals with *S. aureus* colonization but without symptoms of the TSS (B, E, F, G, and H); protein 1 and protein 2 are present in (A), (C), and (D). Cultures (20 ml) grown overnight with shaking were harvested, washed in TE (50 m*M* tris-hydrochloride, 10 m*M* EDTA, *p*H 8.0), and suspended in 4 ml of final solution buffer (62.5 m*M* tris-hydrochloride, *p*H 6.8, 10 percent glycerol, 1 percent SDS, 5 percent β-mercaptoethanol, and 0.02 percent bromophenol blue). Suspensions were then exposed to sonication three times for intervals of 30 seconds; the disrupted cells were then heated to 100°C for 10 minutes. The resulting protein preparations were subjected to electrophoresis on 15 percent SDS-polyacrylamide gel (the electrophoresis buffer contained 1 percent SDS, 3 percent tris base, and 14 percent glycine) overnight at 6-mA constant current. The proteins were transferred to nitrocellulose paper (NCP) by electrophoresis [1 to 2 hours in buffer (14 percent glycine, 0.3 percent tris base, 20 percent methanol)]. The NCP was then soaked for 1 hour in 1 percent bovine serum albumin in TSGAN (50 m*M* tris-hydrochloride, *p*H 7.5, 0.15*M* NaCl, 0.25 percent gelatin, 0.15 percent sodium azide, 0.1 percent NP-40), washed in TSGAN, and incubated overnight in a 1:1000 dilution of TSS patient's antiserum in TSGAN. The NCP was again washed in TSGAN, and 2 μl of ^{125}I-labeled staphylococcal protein A (*11*) in 100 ml of TSGAN was added and incubated for 1 to 2 hours. The solution was decanted, and the NCP was soaked in fresh TSGAN for 1 hour. The NCP was dried and placed next to preflashed Xomat R (Kodak) at −70°C for 24 to 48 hours with intensifying screens (DuPont).

The presence of protein 1 and protein 2 was demonstrated in 25 of 32 (78 percent) isolates obtained from cases and 5 of 20 (25 percent) isolates from controls ($P < .001$ by a two-tailed Fisher's exact test). Figure 1 illustrates the typical pattern of antigenic proteins in *S. aureus* isolates from cases and controls. The presence of proteins 1 and 2 in *S. aureus* strains was associated with 83 percent positive predictive value (*9*) for discriminating *Staphylococcus* strains associated with TSS. The absence of these proteins has a 69 percent negative predictive value for identifying strains unassociated with the disease.

The case-associated isolates initially thought to lack proteins 1 and 2 were reexamined. One isolate possessed only protein 1 whereas the other six isolates thought to be implicated in TSS were indistinguishable from the most common pattern in control strains (that is, neither protein 1 nor 2). Four of these seven isolates were penicillin-sensitive. Since 90 to 95 percent of *S. aureus* isolates from cases of TSS are penicillin-resistant (*10*), some of these seven isolates, although colonizing the patient, may not have been the organisms causing disease. If only penicillin-resistant strains are examined, proteins 1 and 2 are found in 23 of 26 cases and 4 of 18 controls ($P < .001$, Fisher's two-tailed exact test). The positive and negative predictive values become 85 and 82 percent, respectively. Repeated examination of the five isolates (three of which were vaginal isolates) from control patients continued to show both proteins 1 and 2. These data suggest, therefore, that the *S. aureus* associated with TSS may colonize women without causing overt symptoms. Whether such patients have a high risk of subsequently developing TSS remains to be determined.

Our study apparently represents the first successful means of differentiating with reasonable sensitivity and specificity strains of *S. aureus* associated with the TSS. Our results confirm the role of *S. aureus* as the etiologic agent in TSS. Further studies are required for ascertaining whether the characteristic proteins serve only as antigenic markers for identifying toxic-shock strains or actually represent the toxin (or toxins) or

toxin subunits responsible for the clinical manifestations of the syndrome. However, the ability to distinguish staphylococcal isolates should prove of value in examining the epidemiology of TSS and the factors that influence or have influenced the emergence of these strains.

MITCHELL L. COHEN
STANLEY FALKOW*
Departments of Microbiology and Immunology and *Medicine, University of Washington, Seattle 98195*

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Exercise Hyperpnea and Locomotion: Parallel Activation from the Hypothalamus

Abstract. *Unanesthetized decorticate cats walked or ran normally on a treadmill either spontaneously or during electrical stimulation of the subthalamic "locomotor" region. The respiratory response usually preceded the locomotor response and increased in proportion to locomotor activity despite control or ablation of respiratory feedback mechanisms. Respiration increased similarly in paralyzed animals during fictive locomotion despite the absence of muscular contraction or movement. Hypothalamic command signals are thus primarily responsible for the proportional driving of locomotion and respiration during exercise.*

Despite more than a century of study, hypothesis, and debate (*1*), the mechanism leading to hyperpnea, or increased ventilation, during exercise has remained uncertain. The hyperpnea closely parallels the increased metabolic rate resulting from muscular contraction, and therefore arterial CO_2, O_2, and pH remain relatively constant throughout much of the range of moderate exercise. Nevertheless, because of the close relationships between the hyperpnea and metabolic work, many investigators have suggested that chemical receptors variously located in the brainstem (medulla), carotid bodies, lungs, blood vessels, or exercising muscles are the source of stimulation. Others have proposed that neural signals from mechanical receptors in the working muscles or the effects of the exercise-induced increase of body temperature are responsible for hyperpnea. Although such feedback mechanisms exert some influence on respiration under appropriate experimental conditions, no single feedback mechanism, nor any combination of them, has provided a quantitatively adequate explanation for exercise hyperpnea.

An alternative hypothesis is that of a feed-forward mechanism. As long ago as 1913, Krogh and Lindhard (*2*) proposed that neural impulses from the motor cortex that command muscle to exercise also "irradiate" to the respiratory controller in the medulla. Such a mechanism could lead to neurally driven exercise hyperpnea that is proportional to the work performed without the intervention of feedback mechanisms. This hypothesis has never been experimentally validated.

To study the relationship between exercise (locomotion) and respiration in a way in which feedback mechanisms could be eliminated, we used unanesthetized decorticate cats, which Orlovskii (*3*) had shown to walk and run normally on a treadmill. These animals develop locomotion spontaneously as well as during electrical stimulation of the "subthalamic locomotor region" (*3*) and also exhibit "fictive" locomotion in the motor nerves to the legs when the animals are paralyzed with a curare-like agent (*4*).

Our findings support the idea that a neural feed-forward mechanism, originating in the brain at a level above the traditional respiratory centers in the medulla and pons and requiring no feedback mechanisms for its operation, causes both the locomotion and the hyperpnea associated with it.

We decorticated each cat under ether anesthesia, cut the vagus nerves, and denervated the carotid bodies and baroceptors by cutting the carotid sinus nerves. We allowed at least 4 hours for recovery before performing experiments. We measured continuously the carotid arterial pressure, the partial pressure of CO_2 (PCO_2) through a tracheal cannula, and the body temperature, which was kept constant by means of a servo-controlled heater. We quantified respiratory output by measuring the peak integrated electrical activity from the central end of a cut phrenic nerve root (*5*) that was placed in a boatlike bipolar platinum electrode, which we sealed with dental impression material and implanted in the cat's neck. Bipolar electrodes located in both quadriceps muscles allowed quantification of electromyographic (EMG) activity. We then placed the cat's head in a stereotaxic apparatus and suspended the animal over a free-running (not motor-driven) treadmill whose speed could be recorded. A concentric bipolar electrode (*6*) was inserted into the subthalamic locomotor region (*3*) for stimulation (Fig. 1a). At the end of each experiment we perfused the brain in situ with formaldehyde, removed it, and sectioned the diencephalon for histological localization of stimulation sites.

In 9 of 14 experiments, the preparations were successful, and the animals walked spontaneously on the treadmill. An increase in respiration and arterial pressure usually preceded the onset of locomotion, even though there was no increase in end-tidal CO_2 concentration. The cessation of locomotion led to a rapid decrease of both magnitude and frequency of respiration. One animal fortuitously walked spontaneously at two different speeds. The respiratory outputs and treadmill speeds increased proportionately (3229 units per minute at rest, 6735 at a treadmill speed of 14.4 m/min, and 10,815 at 25.9 m/min). Arterial pressure also rose progressively with increasing exercise.

Induction of locomotion by stimulation of the subthalamic locomotor region (*7*) with continuous trains of impulses (30 Hz, 1.0-msec duration) led to similar results. Stimulation caused arterial pressure and respiration to increase promptly, and there was an associated decrease of end-tidal PCO_2 (Fig. 1b). All of these changes preceded the onset of actual locomotion. Ending the stimulation led to cessation of locomotion, a rapid fall in respiration, and a slow decrease in arte-

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