metric nature of the observed pattern precludes explanations based on nonsymmetric instability; the length scales predicted for this experiment from classic symmetric instabilities (*5*) are much larger than the ones observed.

We have also observed sheets in an arrangement where the linear gradient lies below a layer of homogeneous density. In that case, the sheets appear at the top of the gradient, below the homogeneous layer. Density sheets also appear near the bottom of the container when the cylinder speed is changed markedly, and then they decay and disappear as the fluid reaches the new equilibrium rotation speed. Their role in the process of nonlinear, stratified unsteady flow is not yet understood.

The existence of horizontal sheets of density gradient in the laboratory immediately raises the question of the presence of the proposed mechanism in nature. McIntyre (*1*) has suggested that the process could be operative in frontogenesis in the atmosphere, and R. W. Stewart (*6*) has suggested that some mechanism based on the difference between the turbulent transfer coefficients of momentum and density is responsible for the density microstructure observed in freshwater lakes. The present instability would be operative on a scale determined by molecular diffusion, should the molecular coefficients and gradient Richardson number satisfy the instability criterion (Eq. 1); moreover, should the small-scale turbulence in nature result in transfer of momentum and density characterized by constant turbulent transfer coefficients, an eddy Prandtl number could be used in the criterion and the length scales would be increased.

For molecular diffusion, the experiment has demonstrated that sheets appear when σ is greater than 310. Since the proposed instability mechanism is the same for all σ greater than 1, we infer that sheets will appear for all σ between 1 and 310 whenever the critical velocity gradient is exceeded. For example, in a freshwater lake, $\sigma = 7$. The typical temperature gradients of about 0.01°C per centimeter observed in regions of lake microstructure (*6*) lead to instability according to the present theory, if the basic flow is geostrophic and the velocity gradient $\partial v/\partial z > 0.06$ sec^{-1}, a value which is not uncommon (*7*) and which has been observed in regions of microstructure in the Mediterranean Sea (*8*).

Turbulent diffusion coefficients in the instability requirement indicate that the gradient Richardson number must be less than one-fourth the eddy Prandtl number, a criterion that is often met (*9*). Simultaneous measurements of gradient Richardson number and transfer coefficients in the regions of microstructure are required to test the hypothesis that the mechanism exists on these larger scales.

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References and Notes

1. M. E. McIntyre, *Geophys. Fluid Dyn.* **1**, 19 (1970).
2. G. Oster, *Sci. Am.* **213**, 70 (August 1965).
3. M. E. McIntyre, *Geophys. Fluid Dyn.* **1**, 59 (1970).
4. S. A. Thorpe, P. K. Hutt, R. Soulsby, *J. Fluid Mech.* **38**, 375 (1969).
5. P. H. Stone, *J. Atmos. Sci.* **23**, 350 (1966).
6. J. H. Simpson and J. D. Woods, *Nature* **226**, 832 (1970).
7. R. O. Plaisted and W. S. Richardson, *J. Mar. Res.* **28**, 359 (1970).
8. J. D. Woods, *Meteorol. Mag.* **97**, 65 (1968).
9. G. I. Taylor, *Cons. Perm. Int. Explor. Mer Rapp. P.-V.* **76**, 35 (1931). See also H. U. Sverdrup, M. W. Johnson, R. H. Fleming, *The Oceans* (Prentice-Hall, Englewood Cliffs, N.J., 1942), pp. 482–485.
10. I thank M. Stern who first pointed out to me the relevance of the viscous instability to the present experiment, P. H. Stone and F. H. Abernathy for valuable discussions, and J. Duffee, E. Engelmann, and C. Hamaker, of Antioch College, and M. Egan and R. Weller, of Harvard University, for help in the experiments. Supported by a grant NOOO-14-67-A-0298-0011 from the Office of Naval Research to Harvard University.

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Lead Suppression of Mouse Resistance to Salmonella typhimurium

Abstract. *Mice were treated with subclinical doses of lead nitrate for 30 days. Lead-treated mice showed greater susceptibility to challenge with* Salmonella typhimurium *than controls which received no lead. This result confirms the hypothesis that treatment with lead reduces the resistance of mice to bacterial infection.*

According to Chisolm (*1*), "Among the natural substances that man concentrates in his immediate environment, lead is one of the most ubiquitous." The scientific literature is replete with reports on the toxic manifestations, diagnosis, and treatment of lead poisoning. There are also suggestions of subclinical influences of lead on the well-being of man and animals. However, little attention has been directed toward determining the effects and influence of lead on the resistance to bacterial invasion and immunologic reactivity of susceptible hosts. Williams *et al.* (*2*) suggested that lead may inactivate antibodies and thereby interfere with mechanisms whereby man and animals resist infectious disease. Their conclusions were based on the study of an acute, fatal illness in a 23-month-old child with a history of eating paint. Neuropathologic findings were so similar to those seen in acute septicemia that, in spite of concentrations of lead of 0.348 mg per 100 ml of blood, it was assumed that death was due to bacterial agents that were able to grow uninhibited as a result of lead-mediated antibody inactivation. The influence of various environmental pollutants on resistance to disease has been suggested in a number of reports (*3–5*). These include the study of Friend and Trainer (*3*) who described the interaction of an organochlorine pollutant and viral infection in mallard ducklings. Selye *et al.* (*4*) discussed the effect of lead acetate on the susceptibility of rats to bacterial endotoxins, and similar studies have more recently been reported in chicks (*5*).

The study described herein was designed to determine the influence of exposure to subclinical doses of lead on the resistance of mice to bacterial infection. The results confirm the hypothesis that exposure to low concentrations of lead in mice leads to reduced resistance to bacterial infection.

Seventy-five white mice (Swiss-Webster strain) were divided into three groups of 25 each and housed five per cage. The mice were of uniform size and age, weighing 18 to 20 g. Each mouse was given a daily intraperitoneal injection of either soluble lead nitrate or saline for 30 days according to the following paradigm: group I, 100 μg of lead nitrate in 0.5 ml of saline solution; group II, 250 μg of lead nitrate in 0.5 ml of saline solution; and group III (control group), 0.5 ml of sterile saline solution. During the 30-day period of exposure to subclinical doses of lead nitrate (*6*) and saline, no signs of toxicity were observed. Five mice died during this period from other causes: one in group

I, two in group II, and two in the control group.

A strain of *Salmonella typhimurium* (*7*) with limited pathogenicity for mice was selected. The bacterial agent was cultured in trypticase soy broth for 24 hours after transfer from an agar slant. Then the organism was transferred to fresh trypticase soy broth and incubated for 4 hours more. Preliminary titrations of the bacterial agent indicated that a 4-hour culture contained approximately 10^8 organisms per milliliter, as determined by plate counts. The LD_{50} (dose lethal to 50 percent of the animals tested) of the 4-hour culture for normal mice was determined to be $10^{-3.7}$, as calculated by the method of Reed and Meunch (*8*). Each group of five mice was challenged 24 hours after the final injection of lead nitrate or saline with a dilution ($10^{-3.0}$, $10^{-3.7}$, $10^{-4.0}$, $10^{-4.7}$, or $10^{-5.0}$) of a 4-hour culture of *Salmonella typhimurium*. Plate counts of the inoculum were also conducted. Mice were observed daily for signs of illness or death. Confirmation of death by *Salmonella typhimurium* was made by necropsy and culture of organs from the dead mice.

After being given the challenging dose of *Salmonella typhimurium*, 54 percent of the mice in group I but only 13 percent of the mice in the control group died within 7 days (Fig. 1). All of the mice in group II, which had received 2½ times more lead nitrate than the mice in group I, died by the third day after challenge. At the termination of the experiment, the mortality was highest in mice challenged with the lowest dilutions of bacterial culture. The calculated LD_{50} for *Salmonella typhimurium* in the control group was $10^{-3.7}$, whereas that for mice in group I was $10^{-4.7}$. An LD_{50} for the mice in group II could not be determined since all the mice died.

A statistical analysis was carried out to compare the mortality of mice in the three treatment groups during the first 24 hours after challenge. All mice in a group, irrespective of the degree of dilution of the challenge agent, were pooled. The chi-square (χ^2) test indicated a highly significant difference ($P < .01$) between mice in the lead-treated groups and those in the control group. There was also a highly significant difference ($P < .01$) between mice in group I, which received 100 μg of lead nitrate, and those in group II, which received 250 μg.

Continuous exposure of mice to low

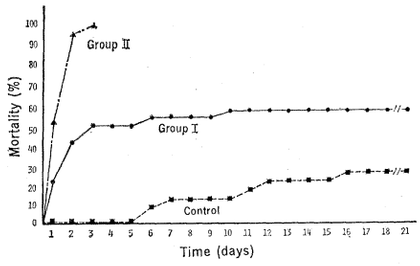

Fig. 1. Accumulated mortality in three groups of mice challenged with *Salmonella typhimurium* var. Copenhagen.

concentrations of lead nitrate during a 30-day period produced no clinical signs of lead toxicity but did indicate enhanced susceptibility to bacterial infection. Such a conclusion is supported by the observation that there was a tenfold difference in the LD_{50} of the *Salmonella typhimurium* organisms between the control and the lead-treated mice. The susceptibility of the lead-treated mice to a strain of *Salmonella typhimurium* with limited pathogenicity was markedly increased.

The very rapid mortality observed for mice in group II (all but one died within 24 hours) raises the question of how lead may increase susceptibility to *Salmonella typhimurium*. Selye *et al.* (*4*) reported that exposure of rats to lead markedly increased their susceptibility to bacterial endotoxin when both were given simultaneously. This enhancement effect probably does not fully explain our results. It is doubtful that quantities of endotoxin sufficient to kill mice could have been present in 0.5 ml of a $10^{-5.0}$ dilution of a 4-hour culture containing approximately 500 organisms. Furthermore, more than 24 hours elapsed between the last administration of lead nitrate and challenge with *Salmonella typhimurium*. A more logical explanation would be that lead interfered with resistance mechanisms in the mice, thereby permitting uninhibited bacterial growth. In the mice receiving 250 μg of lead, lethal quantities of endotoxin could have been quickly produced in the animal with a resultant rapid mortality. In contrast, mice receiving 100 μg of lead were either not so susceptible to endotoxin or were able to restrict somewhat the growth of the bacterial agent. Irrespective of which mechanism was operational, there must have been a reduced resistance on the part of the exposed mice to bacterial multiplication.

The reduced resistance we observed could result from the action of lead on one or several immunologic mechanisms. Interference with phagocytic activity of polymorphonuclear leukocytes has been reported in cases of lead toxicity in man (*9*). Lead has also been reported to bind antibodies in vitro (*2*) and could potentially do so under in vivo conditions. This ability to bind proteins could also interfere with the functional activity of the properdin and complement systems (*10*).

A significant aspect of this study is the effect of subclinical concentrations of lead, since none of the lead-treated mice showed toxic manifestations during the 30-day period of exposure to lead. The increased susceptibility to bacterial infection in the absence of lead toxicity is of paramount public health significance. A large portion of our human and animal population is continuously exposed to appreciable quantities of lead as a result of the presence of this element in air, water, and food. Lead has long been suspected of causing significant changes in normal physiological mechanisms at some point short of clinical disease. Methods are needed for the detection of minimal alterations. The measurement of specific immunologic response mechanisms may provide a sensitive measurement of adverse responses to lead.

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References and Notes

1. J. J. Chisolm, Jr., *Sci. Amer.* **224**, 15 (Feb. 1971).
2. H. W. Williams, W. T. Caraway, W. A. deYoung, *Arch. Neurol. Psychiat.* **72** (No. 5), 579 (1954).
3. M. Friend and D. O. Trainer, *Science* **170**, 1314 (1970).
4. H. Selye, B. Tuchweber, L. Bertók, *J. Bacteriol.* **91**, 884 (1966).
5. R. B. Truscott, *Can. J. Comp. Med. Vet. Sci.* **34**, 134 (1970).
6. Previous titrations of the lead nitrate present in mice of same strain and size indicated that 250-μg quantities administered daily over a 30-day period produced no overt clinical signs.
7. A culture of *Salmonella typhimurium* var. Copenhagen was obtained from J. A. Christopher, Department of Bacteriology, Iowa State University.
8. L. J. Reed and H. Meunch, *Amer. J. Hyg.* **27**, 493 (1938).
9. M. T. Rakkimova, *Gig. Tr. Prof. Zabol.* **12**, 39 (1968).
10. These experimental parameters are presently under investigation in our laboratory.
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