cyclic 3′,5′-nucleotide product with a specific phosphodiesterase yielded 5′-AMP labeled with ^{32}P which was then precipitable with the addition of zinc sulfate and barium hydroxide.
10. R. W. Butcher and E. W. Sutherland, *J. Biol. Chem.* **237**, 1244 (1962).
11. J. T. Potts, Jr., and G. D. Aurbach, in *The Parathyroid Glands: Ultrastructure, Secretion, and Function*, P. J. Gaillard, R. V. Talmage, A. M. Budy, Eds. (Univ. of Chicago Press, Chicago, 1965), p. 53.
12. Purified parathyroid hormone assayed at 2000 to 3000 unit/mg.
13. Purified arginine vasopressin (400 unit/mg) was the gift of Dr. M. Peterson. Purified lysine vasopressin (260 unit/mg) prepared by Drs. R. Guillemin and A. V. Schally was obtained from the Endocrinology Study Section, National Institutes of Health, Bethesda, Maryland.
14. R. F. Pitts, R. S. Gurd, R. H. Kessler, K. Hierholzer, *Amer. J. Physiol.* **194**, 125 (1958).
15. C. G. Duarte and J. F. Watson, *ibid.* **212**, 1355 (1967).
16. R. W. Berliner and C. M. Bennett, *Amer. J. Med.* **42**, 777 (1967).
17. C. W. Gottschalk and M. Mylle, *Amer. J. Physiol.* **196**, 927 (1959).
18. J. Orloff and J. Handler, *Amer. J. Med.* **42**, 757 (1967).
19. H. Wells and W. Lloyd, *Endocrinology* **81**, 139 (1967).
20. We wish to thank Mrs. S. Fedak and C. Woodward for expert technical assistance.

25 October 1967

Fish: Serologic Evidence of Infection with Human Pathogens

Abstract. *Specific antibodies to several bacteria pathogenic to humans were detected in the serums of white perch from surface waters adjacent to heavily populated areas on Chesapeake Bay. White perch from surface waters adjacent to sparsely populated areas were free of such antibodies. We suggest that fish may become actively infected with human pathogens by exposure to contaminated water and may constitute a hazard to public health.*

Many individuals and even communities discharge their excrement into convenient rivers, lakes, or estuaries in spite of the fact that such material frequently contains bacteria capable of causing human disease. It is generally assumed that surface waters so contaminated are naturally purified by dilution, aeration, exposure to sunlight, and antibacterial action of the indigenous microflora. Unfortunately little consideration has been given to the possibility that fish exposed to such contaminated water may become actively infected with pathogenic bacteria and pose a threat to public health: directly through their handling and ingestion, and indirectly by releasing infectious bacteria into waters that may be considered safe according to usual standards of analysis.

Pathogenic bacteria associated with the digestive tracts of man and other mammals have been isolated from many varieties of fish. It has been reported that these bacteria are short-lived contaminants of the surface and gut of the fish, that they derive from human fecal pollution of the aquatic environment, and that they are unable actively to infect the fish (*1*); however, there is evidence that at least some of these bacteria can establish active infection in fish and persist for several weeks or longer (*2*).

Our purpose was to determine whether fish, from surface waters subject to human fecal contamination, had evidence of active infection with pathogenic bacteria associated with such contamination, since actively infected fish could serve as long-term active carriers of human disease as well as short-term passive vectors. We made a serologic survey of serums from white perch, *Roccus americanus*, for antibodies to some of the human pathogens known to be introduced into the aquatic environment chiefly by excrement from man and other mammals. The survey was based on the assumption that antibody in white perch, to a particular human pathogen, would be prima facie evidence that the fish had been actively infected with the pathogen or some closely related species at some time, since fish are known to produce highly specific antibody in response to antigenic stimuli (*3*). The assumption that active infection of the fish is required in order to achieve an antigenic mass large enough to induce production of detectable antibody seems reasonable to us in view of available evidence that experimental injections of large amounts of antigens are required for such production in various fishes (*4*). We should point out that the antibody response of fish to ingested dead bacteria is unknown; it may possibly explain our results.

White perch were selected because they rarely leave the river in which they hatch (*5*); therefore, if they carried evidence of infection, the condition of the river in which they were caught might indicate the source of infecting bacteria. Fish ranging from 10 to 30 cm were netted at random during late summer in rivers flowing into Chesapeake Bay through heavily populated areas and likely to be contaminated frequently, and in sparsely populated rivers of the eastern shore of the bay that are probably rarely contaminated. Each fish was bled by cardiac puncture with a hypodermic syringe, and the

Table 1. Serologic survey of white perch for precipitins against human pathogens. Numbers of fish appear in parentheses.

Source of fish	Fish (No.) with precipitins against pathogen:								
	Past. pestis	*Past. pstb.*	*Salm. para.* A	*Shig. flex.*	*Prot. vulg.*	*Ps. aerug.*	*E. coli* B	*Aero. hydro.*	*Aero. shig.*
				Heavily populated areas					
Potomac River (63)	4	4	1	1	1	1	1	1	
Patuxent River (38)	1	1	1		1	1			1
Severn River (10)						2		1	
Magothy River (25)				1	2	5		*	*
Patapsco River (13)						1			
Back River (26)	3	3		3	3	2	3		
Middle River (16)				1	1	1			
Tolchester Beach (15)			2	*	2	2	2	*	*
				Sparsely populated areas					
Chester River (8)									
Choptank River (25)									
Nanticoke River (7)									
Wicomico River (17)									
				Percentages positive					
	3.0	3.0	1.5	2.3	3.8	5.7	2.3	0.9	0.5

* Not tested.

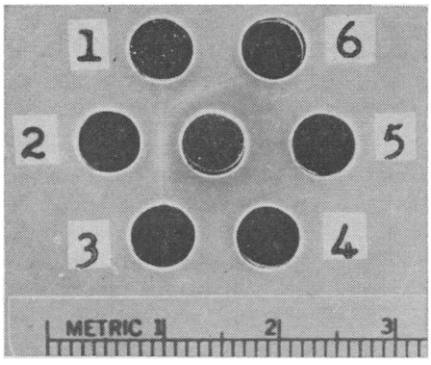

Fig. 1. A gel-precipitin plate. Note typical precipitin bands between serum (center well) and *Pasteurella pestis* (well 1) and *P. pseudotuberculosis* (well 2). Joining of bands indicates that the reacting antigens are identical or closely related. The serum did not react with *Salmonella paratyphi* A (well 3), *Shigella flexneri* (well 4), *Proteus vulgaris* (well 5), *Pseudomonas aeruginosa* (well 6), or any other of the organisms tested.

clotted blood was stored at 5°C until the serum could be tested.

The agar-gel diffusion technique of Ouchterlony (*6*) was used to detect precipitin antibody to the following human pathogens: *Pasteurella pestis, P. pseudotuberculosis, Salmonella paratyphi* A, *Shigella flexneri, Proteus vulgaris, Pseudomonas aeruginosa, Escherichia coli* B, *Aeromonas hydrophila*, and *A. shigelloides*. Cultures of these organisms are maintained at Fort Detrick and their identities were verified (*7*). For preparation of the antigens, each species was cultured in Difco heart infusion broth for 48 hours at 26°C; the bacteria were then disrupted by exposure of the cultures to sonic vibrations for 10 minutes (Raytheon Sonic Oscillator). Finally the cultures were sterilized by filtration, and merthiolate was added at 0.1 mg/ml to maintain sterility. The prepared cultures and white perch serums were stored at 5°C pending the gel-precipitin tests. Agar-gel test plates were prepared with 1 percent Oxoid ion agar No. 2 in physiological saline containing merthiolate at 0.1 mg/ml; they were incubated at 23°C. When present, precipitin bands were easily visible and well defined within 24 hours (Fig. 1).

Precipitin antibodies to each of these human pathogens were detected exclusively in fish netted near heavily populated areas (Table 1). The precipitins were specific in that none of the positive serums reacted with more than one of the organisms, except that eight serums reacted with both *Pasteurella pestis* and *P. pseudotuberculosis*. There two species share most of their antigens (*8*), so that cross-reactions with specific antiserums to either organism are expected. *Pasteurella pestis* was included in the survey, as an indicator of the specificity of the antibodies detected, since it has never been detected in the Chesapeake Bay area and because it shares antigens with several other Gram-negative bacteria (*9*); therefore antibodies reacting with *P. pestis* must be considered nonspecific. The fact that serums reacting with organisms other than *P. pseudotuberculosis* failed to cross-react with *P. pestis* is a further indication of the specific nature of the antibodies involved. The possibilities that the precipitin bands may have been due to nonspecific reactions between antigens and serum lysozyme was ruled out by testing the antigens against lysozyme by the method of Leonard and Thorne (*10*); the results were negative.

Our detection in fish of antibodies to the bacteria that cause human pseudotuberculosis, paratyphoid fever, bacillary dysentery, and a variety of chronic infections is especially ominous since the fish were caught in waters most likely to be contaminated by such bacteria. It is possible that the antibodies were produced in response to infections with bacteria other than those tested, but the close antigenic relations with the human pathogens in question make it likely that the organisms responsible were potentially dangerous to man. In any case, the possibility that fish may become active vectors of human disease, as a result of their infection with pathogenic bacteria in contaminated water, deserves much more attention and study.

WERNER A. JANSSEN
Biological Sciences Laboratory, Fort Detrick, Frederick, Maryland
CALDWELL D. MEYERS
Chesapeake Biological Laboratory, Natural Resources Institute, University of Maryland, Solomons

References and Notes

1. P. J. Glantz and G. E. Krantz, *Health Lab. Sci.* **2**, 54 (1965); J. M. Shewan, in *Fish as Food*, G. Borgström, Ed. (Academic Press, New York, 1962), vol. 2, pp. 443–66; A. Guelin, *ibid.*, pp. 481–502; R. Buttiaux, *ibid.*, pp. 503–19.
2. E. M. Lyayman, *Textbook on the Diseases of Fish* (Izd. Vysshays Shkola, Moscow, ed. 3, 1966), pp. 115–22; W. T. Martin, thesis, Kansas State University (1966).
3. D. L. Everhart and A. M. Shefner, *J. Immunol.* **97**, 231 (1966).
4. M. M. Sigel and L. W. Clem, in *Phylogeny of Immunity*, R. T. Smith *et al.*, Eds. (Univ. of Florida Press, Miami, 1966), pp. 190–7; L. W. Clem and M. M. Sigel, *ibid.*, pp. 209–17; *Federation Proc.* **22**, 138 (1963).
5. R. Mansueti, *Chesapeake Sci.* **2**, 142 (1961).
6. O. Ouchterlony, *Progr. Allergy* **6**, 30 (1962).
7. R. S. Breed, E. G. D. Murray, N. R. Smith, *Bergey's Manual of Determinative Bacteriology* (Williams and Wilkins, Baltimore, ed. 7, 1957); W. H. Ewing, R. Hugh, J. G. Johnson, *Studies on the* Aeromonas *Group* (Communicable Disease Center, Atlanta, Ga., 1961).
8. W. D. Lawton, G. M. Fukui, M. J. Surgalla, *J. Immunol.* **84**, 475 (1960).
9. R. S. Mikhaylova, *Tr. Arm. Protivochumnoy Stantsii* **3**, 143 (1964).
10. C. G. Leonard and C. B. Thorne, *J. Immunol.* **87**, 175 (1961).
11. We thank M. J. Surgalla (Fort Detrick), S. F. Snieszko (Eastern Fish Disease Laboratory, Leetown, W. Va.), and L. E. Cronin (Chesapeake Biological Laboratory) for support and assistance. Contribution No. 342 from the Chesapeake Biological Laboratory.

12 December 1967

Phenol Oxidases of a Lozenge Mutant of Drosophila

Abstract. *Monophenol oxidase (or tyrosinase-A_1) activity appears to be absent from extracts from pupae of homozygous lozenge-glossy* (lz^g/lz^g) *females and* (lz^g/δ) *males of* Drosophila melanogaster. *Diphenol oxidase (tyrosinase-A_2) activity is less in the mutant extracts than in extracts of wild-type Oregon-R pupae of the same age and sex.*

Monophenol oxidase (tyrosinase-A_1), oxidizes tyrosine to 3,4-dihydroxyphenylalanine (dopa) in one of the reactions related to tanning and hardening of the cuticle of the blowfly *Calliphora erythrocephala* (*1*). In addition, both monophenol oxidase and diphenol oxidase (tyrosinase-A_2) have been shown in *Drosophila melanogaster* to be able to oxidize dopa to dopa quinone, which spontaneously polymerizes to the pigment melanin (*2*). Our search for the biochemical aberrations in lozenge pseudoallelic mutants of *D. melanogaster* has demonstrated that the lozenge-glossy lz^g (*g*) has no tyrosinase-A_1 activity and reduced tyrosinase-A_2 activity as tested by either acrylamide-gel electrophoresis or an enzyme assay.

Lozenge-glossy is one of more than 20 known lozenge mutants characterized by less-than-normal development of eyes, female accessory sex organs, claws, and other ectodermally derived organs. The mutant phenotype results from the pleiotropic expression of a gene located at the extreme right locus of four pseudoallelic loci 27.7 map units from the left end of the X-chromosome of *D. melanogaster*. Phenotypically,