

er than to present an extensive series of pH determinations. The animal was alive during the course of readings except in those cases marked †; even here it had ceased breathing only 2-3 minutes before. Some results of other workers on hydrogen ion concentration of the digestive tract of the rat are appended for comparison.

7826 C

Tolerance of *Fundulus Parvipinnis* to Certain Bactericidal Substances.

CLAUDE E. ZOBELL AND NELSON A. WELLS.

From Scripps Institution of Oceanography, University of California, La Jolla, Calif.

The authors have described the bacteriology¹ and the pathology² of an infectious dermatitis of the Pacific killifish, *Fundulus parvipinnis*, and other marine fishes which has seriously handicapped the experimental work^{3, 4} on these organisms. At certain periods during the last 3 or 4 years literally thousands of *Fundulus* have died of the disease in our laboratories and frequently important experiments have been terminated before results of value could be obtained. While the disease is known to occur in nature, probably the fishes are rendered more susceptible to infection by injuries sustained in the collecting nets and by subsequent handling. The elimination of those fishes which are visibly injured, the segregation of the infected, the disinfection of the tanks by chlorination, and the application of other ordinary precautions have been of little or no avail in controlling the epidemics. A hyperthermic treatment consisting of the gradual acclimatization of the fishes to water heated to 32°C. to 35°C. has been of prophylactic as well as of therapeutic value, but this procedure is slow and expensive. Therefore, it is desirable to find a more expedient method for the control of this and similar diseases.

Certain infectious diseases of fresh-water fishes⁵ have been controlled by bathing the fish in solutions of selective germicides. With

¹ Wells, N. A., and ZoBell, C. E., *Proc. Nat. Acad. Sci.*, 1934, **20**, 123.

² ZoBell, C. E., and Wells, N. A., *J. Infect. Dis.*, 1934, **55**, 299.

³ Wells, N. A., *Proc. Nat. Acad. Sci.*, 1932, **18**, 580.

⁴ Wells, N. A., *Physiol. Zool.*, 1935, **8**, April.

⁵ Plehn, M., *Praktikum der Fischkrankheiten*, E. Schweizerbart'sche, Stuttgart, 1924, 179.

this idea in mind experiments were designed to ascertain the tolerance of healthy fishes to such substances. Preliminary work with the inorganic salts of heavy metals removed this class of compounds from further consideration because, while large concentrations are tolerated by the fishes, the metals are precipitated from solution by the constituents of sea water, thereby decreasing their efficacy as germicides. Sumner⁶ found that, due to their insolubility in sea water, certain salts of lead and arsenic in any proportion failed to kill *Fundulus heteroclitus* in 24 hours.

We used *Fundulus parvipinnis* of normal appearance and medium size (5 to 7 cm. in length). They were placed in groups of 2 or 3 in one liter of fresh aerated sea water (salinity ca 34.00‰) at a temperature of 15°C. to 16°C. Larger groups were not used because the vitiation of the water then became a factor which contributed to the distress and even the asphyxiation of the fishes. Different concentrations of various germicides were added to the water. Alcoholic solutions of some were used, but in no case was there enough alcohol to give more than 1%, and control experiments showed that double this amount of alcohol had no perceptible effect upon the fishes. Using new fishes in each case it was noted how long they could remain in the sea-water solutions of germicides before exhibiting signs of marked irritation, discomfort or distress; the maximum exposure they could tolerate and still survive upon being returned to running sea water; and the time required for their death

TABLE I.

Time of exposure to different concentrations of germicides in sea water which caused irritation, discomfort, or distress to *Fundulus*; their tolerance as indicated by longest exposure from which they recovered, and time required for their death.

Germicide	Concentration	Irritation	Tolerance	Death
Mereurochrome	1:100	Immediate	10 min.	35 min.
	1:1,000	15 min.	6 hr.	24 hr.
Acridlavine	1:1,000	45 "	30 min.	1 "
	1:10,000	None	4 hr.	20 "
Crystal violet	1:2,000	30 min.	20 min.	2 "
	1:10,000	—	4 hr.	20 "
Brilliant green	1:1,000	Immediate	?	5 min.
	1:10,000	"	4 min.	30 "
Acridviolet	1:1,000	20 min.	20 "	1 hr.
	1:10,000	None	7 hr.	20 "
Thymol	1:10,000	Immediate	3 min.	10 min.
Chloramine T	1:1,000	"	5 "	20 "
	1:2,000	"	20 "	35 "
Sodium perborate	1:100	"	10 "	30 "
	1:1,000	10 min.	6 hr.	24 hr.
Potassium permanganate	1:1,000	Immediate	2 min.	15 min.
	1:10,000	"	15 "	2 hr.

⁶ Sumner, F. B., *Am. J. Physiol.*, 1907, **19**, 61.

in the solutions. This information is summarized in Table I and is based upon the average response of 4 or more fishes in each series.

The physical response of fishes being bathed in germicidal solutions is not a reliable criterion of their tolerance because there is little or no relationship between the apparent irritability and the toxicity of the germicides. In crystal violet the fishes exhibited no distress even after being in such solutions for a period of time which irreparably injured them, while in mercurochrome they were almost immediately distressed but quickly recovered, after surprisingly long exposures, upon transfer to fresh sea water. Acriviolet and acriflavine seemed to have a semi-narcotizing influence, fishes in these solutions remaining virtually motionless, and usually the first manifestation of distress was the loss of equilibrium in the pre-agonal stage.

With the exception of thymol and brilliant green, all of the substances in Table I were tolerated by *Fundulus* in concentrations which were potentially germicidal towards the etiological agent of the infection, *Achromobacter ichthyodermis*, when suspended in sea water. Experiments with infected fishes revealed that crystal violet, mercurochrome and chloramine T were of no value in checking the infection when the latter was in an advanced stage, probably due to insufficient penetration. In fact, immersion of diseased fishes in germicidal solutions of these 3 substances to the limit of their tolerance seemed to aggravate the pathological processes by rendering the tissue more vulnerable to the invading microbes. Crystal violet and mercurochrome, as well as acriflavine and acriviolet, which appear to be least toxic and most bactericidal of any substances tried, may be useful in preventing the disease if used on healthy fishes shortly after they are exposed to the infection but before it has reached the pre-ulcerous stage of peripheral pallor. Sodium perborate, chloramine T and potassium permanganate are not recommended because of their relative instability in sea water and their pronounced irritability on the fishes.

In general, bathing these fishes in sea-water solutions of the germicides for a duration up to one-half the time reported under the column headed "Tolerance" in the table should provide for a generous margin of safety. The majority of fishes which have been so treated are still alive after several weeks. Also the repetition of this treatment at weekly intervals is tolerated, which shows that there are no cumulative injurious effects.