The Effect of Chlorine on the Motility and Infectivity of the Cercariae of *Schistosoma mansoni*[1]

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THE ACTION of chlorine on the cercariae of *Schistosoma mansoni* is a subject of interest for all professionals engaged in public health work in tropical countries—an interest that has been further enhanced by the present mass-shifting of population to and from endemic areas. This last aspect of the problem has resulted in arousing the interest of the United Nations in other tropical diseases.

Various investigators have reported that chlorine in concentrations varying from one to five parts of chlorine per one million parts of water has a lethal effect on the cercariae of *Schistosoma mansoni*, when these are exposed to the chlorine during intervals of one to fifteen minutes. Complete inactivity was considered a criterion of death of the cercariae. On the other hand, there are investigators who claim that chlorine has no effect on them. In all these studies the source of the chlorine was either sodium or calcium hypochlorite salts, chloramine, or chlorine in the gaseous form. Witenberg and Yofe[2] have reviewed the literature on the purification of water with respect to schistosome cercariae.

The work herein presented confirms the findings of those workers who reported the definite effect of chlorine on the motility of the cercariae. However, as far as the authors are aware, no tests have been made to determine whether such inactivity means their actual death or loss of infectivity. Since the essential factor in the treatment of water with regard to schistosome cercariae is to make sure that these last lose their infectivity, the experiments that follow were made with that particular point in mind.

MATERIALS AND METHODS

Cercariae of *Schistosoma mansoni* were obtained from infected *Australorbis glabratus* collected in the field. Large numbers of the

1. Received for publication July 18, 1944.

2. G. Witenberg and J. Yofe, Investigation on purification of water with respect to schistosome cercariae. Tr.Roy.Soc.Trop.Med.& Hyg., 31:549–571, 1938.

cercariae were accumulated from cistern water or freed from the excreta of snails and other impurities. Estimation of the number of cercariae per cc. of water was made according to the method described by Krakower *et al.*[3]

The chlorine used in this study was high-test calcium hypochlorite (70 percent available chlorine), dissolved in redistilled water. Following the procedure indicated by the "Standard Methods for the Examination of Water and Sewage," a stock solution was prepared, having a calculated concentration of one hundred parts per million of available chlorine, and titrated with sodium thiosulphate prior to the trials carried out on the same day. The desired concentration of chlorine for the various trials was then obtained by adding appropriate proportions of the stock solution to redistilled water. In addition, the chlorine concentrations used in the individual trials were further checked by a colorimetric method with orthotolidine, as indicator, and then compared with a standard chlorine color comparator.

The effect of the chlorine on the motility of the cercariae was determined by direct microscopic examination (magnification, x20) in one cc. volumes of water placed in Syracuse watch glasses. The number of cercariae examined varied from fifty to four hundred and seventy-five per cc. of water; the time required for the cercariae to become completely inactive was recorded. Each determination was repeated several times during a single day or on different days in order to obtain the widest range of values. Control tests, consisting of cercariae in water without chlorine, were always set up with each trial.

The infectivity of the cercariae in chlorine-treated and untreated water was tested on young adult albino rats weighing from forty-five to two hundred and fifty-five grams. A portion of the abdomen of these rats was shaved and the animals were then exposed to the cercariae by dipping them into beakers of water containing the organisms. After an hour of exposure to the cercariae, the rats were removed from the water.

The time at which rats were exposed to the cercariae was that at which microscopic examination of the one cc. portions of water revealed that the organisms were completely inactive. Thus, if the effect of a certain concentration was to be tested, the appropriate amount of calcium hypochlorite was added to the suspension of

3. C. Krakower, W. A. Hoffman, and J. H. Axtmayer, The fate of schistosomes (*S. mansoni*) in experimental infections of normal and vitamin A-deficient white rats. Puerto Rico J.Pub. Health & Trop.Med., 16:346–391, 1940.

cercariae in the beaker, one cc. samples of the water being examined continuously until the cercariae were observed to be completely inactive. The rats were then immersed immediately. All animals exposed to chlorine-treated cercariae and their respective controls were killed four weeks after exposure. At this period, most of the adult schistosomes are found in the liver and a few may be present in the lungs.[4] Hence the liver and the lungs were removed; they were placed in physiological salt solution and sliced into small pieces. Each piece of tissue was then squeezed between two glass plates. This method accelerates the escape of the worms from the tissues into the saline solution. The mixture was then placed in conical flasks and allowed to sediment for thirty minutes. The number of worms there present was then determined.

RESULTS

The action of the chlorine in concentrations found to affect the cercariae of *S. mansoni* may be summarized as follows: the cercariae show weakened movement and sink rapidly to the bottom of the watch glass. While at the bottom of these containers, their bodies and tails move slowly and cease altogether. On the other hand, the cercariae in unchlorinated water are very active and exhibit normal movements.

Table 1 summarizes the effect of the various concentrations of chlorine on the motility of the cercariae. Chlorine concentrations from 1.25 to 0.5 parts per million parts of water caused the inactivity of the organisms in the average time of 2.5 to 10.4 minutes. A con-

TABLE 1

Effect of Chlorine on the Motility of the Cercariae of Schistosoma mansoni[a]

Number of Trials	*Chlorine Concentration in Parts per Million of Water*	*Time in Minutes at Which Cercariae Were Inactive*		
		Minimum	*Maximum*	*Average*
7	1.25	1	4	2.5
13	1.00	1	7	3.2
8	0.75	4	6	4.2
9	0.50	8	18	10.4
3	0.20[b]	..	..	....

[a]Concentrations of cercariae examined varied from 50 to 475 per cc. of water.
[b]No definite effect could be observed at this concentration.

4. C. Krakower, W. A. Hoffman, and J. H. Axtmayer, *op. cit.*

centration of 0.2 parts per million parts of water had no definite effect on the cercariae. Some cercariae kept in water at this concentration for thirty minutes, or longer, sometimes showed inactivation and again suffered no change whatever.

The result of the effect of chlorine on the infectivity of the cercariae of *S. mansoni* is presented in Table 2. Concentrations of

TABLE 2

Effect of Chlorine on the Infectivity of Cercariae of Schistosoma mansoni

Infectivity Trial Number	*Number of Rats Per Trial Exposed to Cercariae in Water*	*Approximate Number of Cercariae in Suspension to Which Rats Were Exposed*	*Chlorine Concentration in Parts per Million of Water*	*Average Number of Schistosomes Recovered per Rat*
1	2	4,000	1.25	0
2	2	2,125	1.25	0
3	2	4,000	1.00	0
4	2	2,125	1.00	0
5	2	4,000	0.75	0
6	2	2,125	0.75	0
7	2	3,000	0.44	0
8	1	3,000	0.22	7
9	2	4,000	0	55
10	2	2,125	0	61
11	1	3,000	0	19

chlorine varying from 1.25 to 0.44 parts per million parts of water rendered the cercariae noninfective; no adult schistosomes were found in the liver and the lungs of rats exposed to cercariae thus treated. However, a concentration of 0.2 had no effect on the infectivity of the cercariae and adult schistosomes were found in one rat after being exposed to cercariae treated with this chlorine concentration. All of the control rats exposed to cercariae not treated with chlorine showed schistosome adults in the liver and the lungs.

SUMMARY

1. Concentrations of chlorine (calcium hypochlorite) in water varying from 1.25 to 0.44 parts per million of water have a definite effect on the infectivity of cercariae of *S. mansoni* when tested on albino rats.

2. A concentration of 1.25 parts per million of water made cercariae inactive and noninfective in a maximum time period of 4 minutes.

3. Cercariae lost their activity and infectivity in a concentration of 0.44 parts per million of water in a maximum time period of 18 minutes.

4. A concentration of 0.22 parts per million of water did not exert a sure effect on the motility of the cercariae. Furthermore, cercariae that apparently showed inactivity at this concentration were still infective to rats.

5. In order to render cercariae of *S. mansoni* noninfective, it is suggested that a minimum exposure period of 20 minutes to the effect of a solution of chlorine, yielding a minimum chlorine residual of 0.50 parts per million of water, may be sufficient.