Some Observations of the Effects of Physical and Chemical Agents on the Cercariae of Schistosoma Mansoni*

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THE ACCIDENTAL observation that the addition of normal salt-solution to rain water in which cercariae are ordinarily collected from infected snails (*Australorbis glabratus*) causes the cercariae to drop and remain at the bottom of the container, led us to investigate this phenomenon more closely and to inquire into the effects of other physical and chemical agents upon cercariae.

In the literature there are few references to such studies for the cercariae of the pathogenic trematodes for human beings. Leiper[1] stressed the need of oxygen for cercariae. He observed that they accumulated near the surface of the water irrespective of exposure to light, but a thin layer of oil or paraffin poured over the surface of the water reduced the life of the cercariae to a few hours. He found that they could survive a temperature of 45° C. but were immediately killed at 50° C. He cites the experience of Conor in Tunis, who noted that bilharziosis was acquired from the waters of the thermal springs at Gafsa, Tozeur and Gabes which have a temperature of 28° C. to 45° C., while the disease was absent in the neighborhood of other springs in Tunis where the temperature ranges from 50° C. to 70° C. Leiper found that weak alkalies had a stimulating, but weak acids an inhibitory effect on the movements of cercariae. He listed acids, acid salts, essential oils and antiseptics which were found to have a lethal effect on cercariae, specifying the strength of the solutions.

Faust and Hoffman[2] found that the cercariae of *S. mansoni* were liberated from the snails almost exclusively between 9 A.M. and 2 P.M., particularly when exposed to direct sunlight. They describe the movements of the cercariae, their upward active ascent, caudal ends foremost, and their passive descent to the bottom of the container. They state that "after 18 to 24 hours of freedom in the water the cercariae begin to congregate in increasing numbers at the bottom of the container.....(where) they tend to crawl rather than swim. They become reactivated, however, by mechani-

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cal agitation of the water. The forms which settle down to the bottom, although still viable, would not be in a favorable position to come in contact with the skin of persons using the water and would therefore appear to be less likely to produce infection than younger specimens which have just been discharged from the snail." These authors also determined the thermotactic reactions of the cercariae. When ice was packed about the bottom of the cylinder containing thousands of cercariae, most of them accumulated near the top. The cercariae were immobilized if they penetrated into the cold zone, but remained viable there and became reactivated when the ice pack was removed. As to viability, Faust and Hoffman found that about 90% of the cercariae died within 30 hours after emission from the snails.

Isobe[3] observed that the temperature of the water controlled the escape of the cercariae of *Schistosoma japonicum* from their molluscan host. At 15° C., no cercariae escaped. At 18 to 19° C., it was necessary to keep the snails for four hours before any cercariae were liberated; at 24° C., two hours only were necessary. Tanaka[4] observed, even as Conor in Tunis did, that the temperature of bath water used by the Japanese is 43 to 47° C., the optimum being 45 to 46° C. In such warm water, he believed the cercariae of *S. japonicum* are unable to infect the human body. If, therefore, bath water is cooled by well water, not by river water, or if the heating of river water should have been continued for more than 5 minutes before the person bathes, there would be no danger of infection. Bettencourt[5] found that a soapy solution, 2, 1 and 0.5 parts per 1000, permitted cercariae of *Schistosoma haematobium* to survive 2 hours 5 minutes to 3 hours 30 minutes,—a sufficient length of time to allow the organism to penetrate the definitive host.

Tubangui and Masiluñgan,[6] in a study of the cercaricidal property of the sera of vertebrate animals, found "that some cercariae were very susceptible to salt solutions." Based on the work of Culbertson and Talbot[7] and Culbertson[8] on the cercaricidal action of sera, the above mentioned authors found also that a variety of sera, including that of man, monkey and guinea pig, were cercaricidal for *S. japonicum*. The sera of cats and rabbits, however, were not.

For the present studies the following procedure was generally adopted. The infected snails were removed from their aquaria during the morning, washed in rain water to remove mucus and débris, and placed in a large beaker (1- or 2-litre capacity) partly filled with clear rain water. The beaker was left uncovered and placed near the window in full daylight. Early in the afternoon the snails were

removed. The water, now heavily charged with cercariae, was poured into another clean beaker to get rid of residue from the snails. This was then treated in various ways, depending upon the type of experiment. If some chemical agent was used in the investigation, the water was lightly stirred to obtain uniform distribution of cercariae, was then measured in a cylinder and poured into smaller beakers. The desired concentrations of the agent were slowly added to these, and by gentle stirring rapid uniform diffusion was obtained. Final observations were made in clean test tubes to which 10 or 15 c.c. of the treated or untreated water containing the cercariae were added. Untreated control test tubes with cercariae were always concomitantly employed.

At first, watch glasses were used and observations were made with the dissecting microscope. It was found, however, that test tubes were greatly superior for studying the cercariae. By employing the strong light and to a degree the heat of a microscope lamp filtered through a flask with copper sulphate solution, many test tubes could be examined rapidly and the state of the cercariae quickly ascertained. If the cercariae were alive and active, their mass movement towards the surface was quickly secured by placing the test tube against the flask so that the upper fluid levels were exposed to the light. The activity or inactivity of the cercariae at the bottom could also be examined by directing the light accurately. For additional checks on the condition of the cercariae, either a small hand lens was employed and the dispersed cercariae examined after the test tube had been shaken, or the sediment at the bottom was examined with an ordinary microscope by pipetting some of it out of the test tube and placing it on a slide.

When left at room temperature in rain water, cercariae generally survive for 24 to 48 hours, though occasionally a small percentage may live for as long as 72 hours. With progressive increase in the time after emission from the snails, more cercariae die. Even while quite active, if left undisturbed, the cercariae tend to go to the bottom of the container; but the slightest stimulus, whether mechanical, light or heat, will cause them to rise *en masse* towards the surface. Cercariae, however, react this way in decreasing numbers as they age. Progressively more remain at the bottom and there die.

It has been found that, as a general rule, either in association with the natural death of the organism or as the result of injury the following stages may be regarded as indicative of progressive degrees of devitalization. Of course, if the injury is pronounced, either marked

devitalization or death of the cercariae will occur directly, without intermediate stages:

First stage: The cercariae are active and mobile, but fail to rise to the top of the fluid, although they may ascend for a shorter or greater distance from the bottom.

Second stage: The cercariae are still active and mobile but their activity is confined largely to the bottom of the container, and they rise, at most, for only a very short distance.

Third stage: The cercariae now remain permanently at the bottom, but exhibit frequent sharp twitching movements of body and tail.

Fourth stage: The movements of the cercariae are even more restricted, and consist only of a nodding motion, whereby the body slightly contracts and the tail is flexed on the body.

Fifth stage: There is practically complete immobility except for faint twitching of body or tail detectable only with the microscope, although one or more of the flame cells continue to be active.

Sixth stage: Only one or more flame cells may be seen to function, the organism otherwise being entirely motionless.

Death and disintegration of the organism with separation of the tail from the head soon follows. Prior to death the secretions from the anterior and posterior glands may escape as a cloud from the ducts. After death the cercariae adhere to the walls and bottom of the tube. Small air bubbles accumulate next to the cuticle of the organism, portions of the inner mass are extruded and, finally, complete granular disintegration occurs. With the increase in disintegrated cercarial products the population of ciliated protozoa generally increases.

In the recovery phase from any injury, the above stages towards full activity and motion are often reversed. This, of course, need not include *all* stages, as the degrees of previous injury may vary. Such recovery may be considerably delayed after removal from an injurious agent, as, for example, heat or cold.

There is reason to believe that different lots of cercariae react in a markedly different manner towards injurious agents, with regard to duration of life. Although not yet put to direct experimental test, it is also felt that cercariae collected at different seasons of the year show such variation in behavior.

EFFECT OF COLD

Test tubes containing cercariae in 10 to 15 c.c. of water were placed in an ice chest where the temperature averaged 5 or 6° C.

TABLE 1

Experiment I: *Effect of Cold upon Cercariae*

Duration of refrigeration (in hours)	*Remarks*	*Survival After Removal from Cold*
6	All cercariae are very active and swarm to the top when stimulated by light after removal from cold. At the end of 36 hours there are many that rise to the top, some active at the bottom, many dead.	All dead within 48 hours.
18	As above except that after 32 hours at room temperature quite a number of cercariae are still active and will rise to the top.	Practically all dead within 36 hours.
24	As above.	All dead within 42 hours.
42	Five to ten minutes after removal from ice chest all cercariae are active at the bottom but only rise for a short distance. Within an hour they are still unable to rise to the top, but practically all are capable of doing so after 8 hours at room temperature. After 24 hours all are quite active and mobile, rising to the top. After 30½ hours many are dead at the bottom, some are feebly active at the bottom, some are active and rise to the top.	Practically all dead within 34 hours.
54	The cercariae are immobile at first. Three to five minutes after removal from cold the cercariae present wriggling or twitching movements at the bottom. Fifteen minutes after removal they will rise for a short distance when stimulated by light with lashing movements in a plane parallel with the surface as they themselves are orientated in the same plane. After 12 hours all cercariae are very active and rise to the top. After 18½ hours many cercariae are at the bottom, some with feeble twitching or nodding movements, others are dead. The others rise readily to the top.	All dead within 36 hours.
66	More or less as in the 54 hour period.	All dead within 31 hours.
76	More or less the same as in the 54 hour period.	All dead within 27 hours.
90	One or two minutes after removal from the ice-chest the cercariae are immobile. A minute or two later they begin to twitch. One-half hour later they are all active at the bottom but only few of them attempt to rise. After 7 hours a large number continues to be active at the bottom but many rise to the top after 24 hours. Several rise rapidly to the top, some present feeble movements at the bottom, the others are dead and autolyzed.	All dead within 30 hours.
103	Many are dead immediately after removal from the ice-chest and with gentle shaking of the test tube their heads separate from the tails. Twelve hours later of those that survive a number will rise for a short distance from the bottom but only few reach the top. The movements of the cercariae are peculiarly "ataxic." After 17½ hours there are few that rise to the top and few feebly active at the bottom; others are all dead.	Practically all dead within 24 hours.
114	More or less as in the 103 hour period.	Practically all dead within 24 hours.
126	More dead when first examined after removal from ice-chest. Very few are alive 12 hours later.	All dead within 24 hours.
138	As in the 126 hour period. Many cercariae are alive after 12 hours at room temperature.	Practically all dead within 24 hours.

TABLE 2

Experiment II: *Effect of Longer Refrigeration on Cercariae Than in Experiment I*

Duration of refrigeration (in hours)	*Remarks*	*Survival After Removal from Cold*
115	Most of the cercariae are alive immediately after removal to room temperature. They never rise to the very top subsequently, even when they are fairly active at the bottom.	Most are dead within 24 hours.
140	As in the 115 hour period.	Most are dead within 24 hours.
162	Fifteen minutes after removal from ice-chest many cercariae are active, rising in many instances half the length of the fluid column. After 21 hours there is a heavy sediment of dead cercariae but many rise spontaneously for a distance, some even reaching the top. In 24 hours there are still some alive and one or two fairly active.	All dead within 36 hours.
185	Heavy sediment of dead ones at the bottom after removal from cold. Nine hours later several very active cercariae and a number of feebly active ones. In 12 hours some six or eight rise spontaneously for a distance and there are only very few additional live ones at the bottom.	Practically all dead within 24 hours.
210	Relatively few live ones with at best twitching movements in 2 hours. Some 12 alive and fairly active at the end of 8 hours.	Only one or two live ones at the end of 24 hours.
235	As in the 210 hour period.	All dead within 24 hours.
258	Only about a dozen survive refrigeration.	Practically all dead within 12 hours.
283 307 330	Only very few survive. Some 20 alive in the 283 hour period, 12 to 18 in the 307 hour period, and only about 3 in the 330 hour period.	

They were removed periodically from the cold. Control test tubes were left at room temperature from the outset of the experiment. It had been found previously that freezing rapidly killed the cercariae. Where freezing was avoided, however, most of the cercariae survived the first 4 to 6 days of refrigeration, whereas all in the control tubes died within 48 hours. (Tables 1 and 2.) Subsequently, mortality increased under refrigeration, until about the 12th or 13th day when only a few cercariae survived.

When chilled, the cercariae are immobilized and rapidly drop to the bottom of the container. When removed to room temperature, those that survive quickly become active, their twitching movements heralding full activity, though they are incapable of reaching the surface. Finally they may assume full normal activity. They may then survive at room temperature for almost as long a time as the original control cercariae, though, in general, those that survive a longer period of refrigeration are less apt to live for 24 hours at room temperature after removal from the cold. This should be compared with the marked reduction of survival time after cercariae have been exposed to the effects of heat.

That the cercariae surviving refrigeration are capable of completing their life cycle in the mammalian host was shown by the fact that rats could be successfully infected with them. Thus, in the first 4 days after refrigeration, which most cercariae survive, heavy infections were obtained in rats exposed to them after they had recovered their activity at room temperature. Adult worms, worm pigment and eggs were demonstrable in rats infected with cercariae that had been refrigerated for 5 and 6 days. After that period of time, the numbers of surviving cercariae were inadequate to produce obvious infection. Only a few adult worms were identified in the liver of a rat that had been exposed to cercariae refrigerated for 8 days.

EFFECT OF HEAT

Test tubes with 10 to 15 c.c. of water containing cercariae were placed in a water bath kept at constant temperature. They were removed periodically from the bath, generally after ¼, ½, 1, 2, 3, 4, 5, 7 and 8 hours, respectively. They were subsequently examined periodically at room temperature. Control test tubes were left at room temperature from the very outset of the experiment.

At *32° C.* all cercariae survive the heating and subsequently live as long as the control cercariae at room temperature. All are alive

and active 24 hours after the beginning of the experiment and all are dead including the control cercariae within 42 hours.

At *34° C.* precisely the same results are obtained as at 32° C.

At *36° C.* there is no effect upon the cercariae exposed for ¼ or ½ hour. Those exposed for 1 and 2 hours, respectively, at that temperature, while they reveal little immediate effect, i.e., for the first 7 hours, present a much higher mortality at the end of 20 hours as compared with the controls. From the third hour of exposure onwards, progressively greater numbers of dead and sluggish ones are found immediately after removal from the bath. While a small percentage survive for 20 hours after 3 hours of exposure in the water-bath, those that had been exposed for 6, 7 and 8 hours, respectively, are practically all dead.

At *38° C.* more or less similar results are obtained as at 36° C. This lot of cercariae was "hardier" than those in the previous experiment; more of the control cercariae survived for 24 hours. There seemed to be some evidence that even 15 minutes of exposure at 38° C., while without any immediate effect, was associated with a higher mortality of cercariae at the end of 24 hours.

At *40° C.* the late effects of brief exposure to heat are more manifest. Thus, after ¼ hour of exposure all cercariae remain alive and active for the first 8½ hours, but practically all are dead within 19 hours; whereas most control cercariae are alive and active at the end of that time. After ½ hour of exposure all cercariae are alive and active for the first 5½ hours. Progressively more die in the next 3 hours, and most are dead after 19 hours. From the first 4 hours after exposure at 40° C., progressively more cercariae are found to be dead after removal from the bath and progressively more die at room temperature. Most of the cercariae are dead after 6 and 7 hours of exposure at 40° C. All are killed after 8½ hours of exposure.

At *42° C.* many cercariae are killed after ¼ and ½ hour of exposure. Of those that survive some continue to be alive and active as long as the controls. After 1 hour, about 50% are killed, while after 2 hours most are dead.

At *43° C.* about 25% of the cercariae are killed after 15 minutes of exposure. Of those that survive, some remain alive and active as long as the controls. About 50% of the cercariae are killed after ½ hour of exposure. Most of the cercariae are killed after 1⅓ hours of exposure. Only on rare occasions do a few sluggish ones survive.

At *44° C.* a moderate number survive 15 minutes of exposure (about 25%) and many of these remain poorly active at the bottom,

never obtaining full active mobility. After ½ hour of exposure a small percentage survive but never exhibit more than poor activity at the bottom of the test tube.

At *45° C.* all cercariae are killed after ½ hour. Although most appeared dead after removal from the bath following 15 minutes of exposure, a fair number showed twitching movements 3 hours later and a few were still alive and quite active at the bottom 24 hours later.

It may therefore be concluded that the thermal death point for cercariae is about 6 hours at 40° C., 2 hours at 42° C., 1⅓ hours at 43° C. and ½ hour at 45° C.

It is interesting to note that even brief exposures at lower temperatures such as 36° C. are not without their influence on the ultimate survival time of cercariae, even though they may appear quite normal for a few hours after removal to room temperature. This of course would suggest that the cercariae possess a reserve of energy which probably lasts for 24 to 48 hours under ordinary circumstances but is reduced by exposure to heat. Another interesting feature is that at higher temperatures the organism is paralyzed by the heat and if it is not killed may only recover partially or completely after a considerable lapse of time at room temperature.

EFFECT OF DARKNESS

To determine whether the absence of light as well as other stimuli would lengthen the survival time of cercariae, test tubes were placed in a tin container with tight-fitting cap which had previously been covered with black mat. The tin container was in turn placed in a larger cardboard box, the interior of which had been draped with black mat. The box was placed in a dark cupboard.

Result: No difference in survival time was found as compared with controls.

EFFECT OF MIXED LIGHT

Five 200 watt bulbs were used as sources of light. They were arranged to give maximum illumination to all parts of the fluid column in the test tubes placed in a rack. Two electric fans were employed to dissipate the heat. The temperature of the fluid in the test tubes was on the average about 31° C., never above 33° C. as compared with a room temperature of 27° C. Owing to wind currents set up by the fans there was a certain amount of shaking movement imparted to the test tubes.

Result: The first two hours of exposure to strong mixed light are without appreciable effect upon cercariae. After 6 hours of exposure most cercariae die within 24 hours whereas the controls lived for 36 hours. Longer periods of exposure are associated with a higher mortality so that most cercariae are dead when they are removed from the light after 18 hours. This effect can scarcely be attributed to the elevated temperature but appears rather to be associated with the constant stimulus of strong light. The first reaction of cercariae to a strong light source is active swarming towards it. After a short while the cercariae are distributed. Finally they settle towards the bottom at first remaining active about the middle third of the tube, then settling close to the bottom where they exhibit less sustained and less intense movements.

EFFECT OF ULTRAVIOLET LIGHT

Cercariae were exposed in a thin layer of water in a large culture dish to an alpine lamp operating at 5 ma. at a distance of 75 cm. At that distance, a mild erythema of the skin was obtained in 5 minutes and a fairly intense erythema in 20 minutes. After the period of exposure the cercariae were transferred to test tubes.

Results: After 15 minutes of exposure the cercariae are apparently temporarily injured but are practically normal one half hour later and survive as long as the controls.

Twenty minutes of exposure to ultraviolet light permanently injures the cercariae. Some are killed directly. The others fail to rise, revealing either feeble or moderately active movements at the bottom. Many of these, even with restricted movements, live as long thereafter as the controls.

Forty-five minutes of exposure to ultraviolet light kills all cercariae outright.

EFFECT OF SUNLIGHT

Test tubes with cercariae were exposed to full sunlight. Whereas the external temperature in the sun rose infrequently for a very brief spell to 42° C., it never exceeded 34° C. within the fluid of the test tube. Even one-half hour of exposure to strong sunlight severely injured the cercariae more or less in the same manner as 20 minutes of exposure to ultraviolet light. Many cercariae were killed after 45 minutes of strong sunlight and most were dead after 1 hour. Even when the afternoon was relatively cloudy the cercariae were severely injured and many were killed after one hour of exposure.

In one experiment the mouths of the test tubes (Pyrex) were covered with black mat and exposed to full and almost continuous sunlight and the external temperature in the sun never rose above 37° C. One hour of exposure was without much appreciable immediate effect, although a greater percentage was dead the following morning, as compared with the controls. After 2 hours of exposure there were evidences of mild injury, many cercariae being permanently down at the bottom but still quite active 3 hours later. Many were dead the following morning. When on the other hand the test tubes were completely covered with black mat leaving only their mouths exposed to direct sunlight, the cercariae were essentially unharmed. It is assumed that the light entering the mouth of the tube was largely absorbed by the black background shielding its sides.

Under the conditions of these experiments direct sunlight is injurious to cercariae. How much of this is due to the direct heating effect of the sun and how much to ultraviolet radiation must be left for more detailed experimental procedures.*

EFFECT OF CHANGES IN pH

To avoid the effect of buffers which might introduce other factors to be discussed later, ranges of pH from 0.6 to 10+ were adjusted with normal solutions of hydrochloric acid and sodium hydroxide respectively employing the standard range of dyes. It was found that methyl red was highly toxic and instantly killed the cercariae. The other dyes were without apparent effect.

Results:

pH values below 3 kill cercariae immediately.

pH 3.2 causes the cercariae to drop to the bottom. There they continue to be active for about half an hour without responding to a strong stimulus of light. They are dead within an hour.

pH 3.6 to 3.8 causes the cercariae to drop to the bottom. At first they are fairly active but subsequently more twitching and nodding movements are noted. Practically all are dead within 2 hours.

pH 3.9 as 3.8 but it takes a little longer time for the cercariae to

*Professor Donald H. Cook kindly prepared a chart demonstrating that a 2 mm. layer of Pyrex glass will transmit about 61% of rays of 3200 A.U., 40% rays of 3100 A.U. and 12% of rays of 3000 A.U. It is realized that more exacting experimental procedures will have to be adopted to determine: (1) The range of effective ultraviolet wave lengths which will kill the cercariae, (2) Under experimental conditions more closely approximating the natural habitat of cercariae and by elimination, how much the heating effect of sunlight has to do with destruction of the cercariae and how much its effective range of ultraviolet radiation.

drop permanently to the bottom. Practically all are dead within 3 hours.

pH 4.2 injures the cercariae. They are made to rise only sluggishly with intense stimulation by light at the end of 2 hours. At the end of 3 hours all are permanently at the bottom and many are dead.

pH 4.6 to pH 10+ (probably not exceeding 11.0) had no effect on cercariae.

Alkaline values estimated to be above pH 11.0 killed the cercariae.

Cercariae are therefore unharmed within a pH range of 4.6 to approximately 11.0.

EFFECT OF DEPRIVATION OF OXYGEN

Test tubes with cercariae were placed in an anaerobic jar. The air was replaced with hydrogen.

Result: The cercariae were alive and active for the first 5 hours but all were dead at the end of 6¾ hours. The cercariae in control tubes were alive and normal. It is probable that there was sufficient oxygen in solution to maintain the life of the cercariae for as long as it did.

When the air was replaced by carbon dioxide, the cercariae were alive for the first 10 minutes but when next seen 4 hours later, all were dead. The effects of different grades of oxygen tension on cercariae were not determined.

EFFECT OF SALT AND OTHER SOLUTIONS

In general it required about 10 minutes to half an hour before the effects of a given solution became apparent.

Sodium chloride: Hypotonic solutions under 0.6% (0.1 M) were without any effect. 0.6 (0.1 M) to 1.0% (0.17 M) solutions caused the cercariae to drop permanently to the bottom remaining fairly active there or with more restricted movements. They generally survived as long as the controls.

Hypertonic solutions 1.5% (0.256 M) and over rapidly killed the cercariae.

Glucose: Hypotonic solutions under 2.7% (0.15 M) were without any effect. 2.7 (0.15 M) to 5.4% (0.30 M) caused the cercariae to drop permanently to the bottom with greater restriction of movements in the solutions of higher concentration.

With isotonic saline and glucose solutions it was found that most of the cercariae could be restored to normal activity by exchanging

the solutions for rain water. This was possible within the first 8 hours, at least. Later, the altered state of the cercariae resulting from the isotonic solutions appeared to be irreversible. Thus, where cercariae in isotonic salt solution had been refrigerated, although they survived for a number of days, their activity could not be restored by changing the fluid for rain water after removal from the ice-chest. If anything, such process appeared to hasten disintegration.

The restricted ability of cercariae to infect a mammalian host, once they have been caused to sink towards the bottom of the container by the addition of isotonic solution (even though they are still active there), is demonstrated by the following experiment:

Two rats were exposed to large numbers of cercariae in isotonic salt solution after the cercariae for the most part had dropped towards the bottom of the container. The rats were left in this solution for about an hour. They were killed 39 days later. Only 9 adult schistosomes were counted in the whole of the liver of one of the rats and only 10 in the other. In view of the high degree of cercarial concentration to which the rats were exposed, this would indicate that only a very small percentage of these were able to penetrate and mature in the host. The reason for this is, perhaps, to be sought not so much in the actual effects of the salt solution on the penetrability of the cercariae through the skin, as it is that restricted movements of the cercariae diminish their chances of contact with the host. In this connection it is interesting to note the statement of Faust, Jones and Hoffman that

> careful observations and inquiries have demonstrated that cercariae are[9] apparently unable to attack and penetrate the human skin as long as these cercariae are any considerable distance below the surface film. For persons wading in the water the earliest indications of infection are associated with the part of the body at or above the water level, usually the lower extremities, while individuals bathing or swimming in the infected pools more usually experience attack and invasion by the cercariae over the entire body.

Ringer's and Locke's fluids: These cause cercariae to drop to the bottom. But whereas in Ringer's fluid they remain fairly active, in Locke's solution they are practically motionless, although one can convince oneself that they are alive by microscopic examination.

Monosodium acid phosphate ($NaH_2PO_4 . H_2O$): Up to 0.6% (0.043 M): No effect.

0.8% (0.057 M): All cercariae permanently descend to the bottom in 15 minutes. For the next 1½ hours movements are restricted and are of twitching and nodding character. Four hours later, all are motionless.

1.2% (0.086 M): Most cercariae are motionless at the bottom within 15 minutes.

2.0% (0.144 M): Kills cercariae within 15 minutes.

2.0% $NaH_2PO_4 .H_2O$ does not crenate red blood cells.

Disodium acid phosphate ($Na_2HPO_4 .12H_2O$): 0.6% (0.016 M): Within one-half hour all are permanently at the bottom and continue to be active there for the next 2 hours. After 3½ hours many are motionless, a number move pretty briskly or merely twitch.

0.8% (0.022 M): More or less as in 0.6% but less active at the bottom for the first 2 hours and more are dead 3½ hours later.

1.2% (0.033 M): The injurious effects are more marked than in 0.8%.

1.8% (0.05 M): All are and remain motionless at the bottom within half an hour.

1.8% solution is hypertonic to red blood cells. 1.2% solution is isotonic to red blood cells. All the solutions were cloudy and flocculent.

Potassium chloride: 0.1% (0.013 M): No effect.

0.2% (0.027 M): Within the first hour most cercariae react normally. One hour later most of the cercariae are permanently at the bottom but are fairly active. Three hours later, (i.e. 5 hours after beginning the experiment) all cercariae are dead.

0.4% (0.054 M): All cercariae are permanently at the bottom but fairly active within the first hour. In the next hour some are dead, the others merely twitch or nod. All are dead within the next 1½ hours, (3½ hours after onset of experiment.)

0.6% (0.08 M): Most of the cercariae are motionless at the bottom within 1 hour. All are apparently dead within 2 hours.

0.8% (0.107 M): All are killed within 1 hour.

1% KCl is not hypertonic for red blood cells.

Monopotassium acid phosphate (KH_2PO_4): 0.4% (0.029 M): All cercariae are permanently at the bottom, many motionless, the others twitching after one hour. All are motionless within 6 hours.

0.6% (0.044 M), 0.8% (0.058 M) and 1.0% (0.073 M): As above except that practically all are motionless within 3 hours.

1% is not hypertonic for red blood cells.

Dipotassium acid phosphate (K_2HPO_4): 0.3% (0.017 M): Cercariae behave normally for the first hour. They are chiefly active near the bottom 1½ hours later; and all are dead 4 hours later, (total duration is 6½ hours).

0.6% (0.034 M) and 0.8% (0.046 M): All cercariae are perma-

nently at the bottom within 1 hour. Some are motionless, others are poorly active. Practically all are motionless within 3½ hours and all are dead in 6½ hours.

1.2% (0.068 M) to 2.0% (0.114 M): Practically all are motionless very soon after being placed in the respective solutions.

1.8% is hypertonic for red blood cells. There was flocculent clouding of the solutions.

Calcium chloride (anhydrous): 0.1% (0.009 M): No effect.

0.3% (0.027 M): The cercariae are normal within the first hour. During the second hour there is a heavy sediment of poorly active ones. In the next two hours all cercariae settle permanently to the bottom remaining either fairly or slightly active with some dead. Many cercariae are dead 9 hours after the onset of the experiment.

0.5% (0.045 M): More or less as in 0.3%.

0.8% (0.072 M): All cercariae rapidly drop to the bottom where they exhibit either rapid twitching or nodding movements. The percentage of dead ones increases rapidly so that most are dead within 9 hours.

1.2% (0.081 M): As above, but all are dead within 5 hours.

1.5% (0.135 M): The cercariae are killed almost instantaneously.

1.2% is mildly hypertonic for red blood cells.

Magnesium chloride ($MgCl_2 .6H_2O$): 0.1% (0.004 M) to 1.2% (0.059 M): No effect.

1.3% (0.064 M): Causes the cercariae to drop permanently to the bottom where they generally remain quite active for the first 4 hours. Movements are more restricted subsequently.

1.8% (0.088 M): Cercariae drop permanently to the bottom almost at once where their movements are restricted to twitches and nods. The mortality increases thereafter.

2.0% (0.098 M) to 2.5% (0.123 M): All cercariae rapidly sink to the bottom where they soon become motionless. Flame cell activity can be recognized in the ones in 2.0% for the next few hours, but not in the ones in the 2.5% solution.

2.5% $MgCl_2$ is without effect upon red blood cells.

Disintegration of the dead cercariae was practically absent suggesting that the magnesium chloride might have interfered with the process of autolysis.

Barium chloride ($BaCl_2 2H_2O$): 0.05% (0.002 M): For the first 2 hours fewer cercariae remain active at the surface and more sink permanently to the bottom. Most of the cercariae are killed within 4 hours.

0.1% (0.004 M) to 0.75% (0.03 M): All cercariae rapidly drop to the bottom where practically all are killed within 4 hours.

0.75% (0.03 M) to 1.8% (0.073 M): All are killed within 2 hours.

2.0% (0.082 M): All are killed well within an hour.

2% $BaCl_2$ is isotonic for red blood cells. The higher concentrations of $BaCl_2$ were cloudy.

Urea: 0.02% (0.003 M) to 0.12% (0.02 M) solutions were without effect upon cercariae.

A review of the effects of salts and other solutions on cercariae reveal certain interesting facts. It will be noted that the effect of sodium chloride first becomes evident at 0.6% which is approximately the concentration of sodium chloride in plasma, (570 to 620 mgms. per 100 cc.). Therefore, whole blood or serum in itself would cause considerable restriction in the mobility of the cercariae *if one excludes the cercaricidal principle.* In fact it is the only agent present in sufficient concentration in blood or lymph to produce this effect, as will be shown later. It is interesting to note, however, that once the cercaria has developed in the host the schistosome can be kept alive for a considerable length of time in isotonic salt solution at room temperature but soon dies when placed in water.

There is quite a wide range of safety as far as some of the other elements are concerned. Glucose is present in serum to the extent of about 0.1 to 0.12 grams per 100 cc., whereas the effects on cercariae are only noted with concentrations of 2.7%. Both here and with the 0.6% sodium chloride the effects would appear to be due to the tonicity of the solutions. Urea even to the extent of 0.12 grams per 100 cc. was without effect on cercariae, while in serum its concentration is 0.019 grams per 100 cc. Potassium appears definitely toxic wherever its concentration rises to 0.1 gram or more per 100 cc. Thus a 0.1% solution of potassium chloride containing 0.052 grams of K is without effect upon cercariae, while a 0.2% solution with 0.104 grams of K is toxic. By the same token 0.4% KH_2Po_4 with 0.11 grams K and 0.3% K_2HPo_4 with 0.13 grams of K are both toxic. It is to be recalled that serum potassium is 0.018 to 0.022 grams per 100 cc., i.e., 10 times less than the above mentioned concentrations. Since, however, the value of K for whole blood is 0.15 to 0.25 grams per 100 cc., it is conceivable that with lysis of red cells and the release of available K ions toxic levels at least in vitro might be reached.

No satisfactory reason for the differences in behaviour of 0.6% and 0.8% monosodium acid phosphate can be given. Both are about

pH 5 well within the safety zone of activity for cercariae. Neither do the tonicity of the solutions nor their concentrations of sodium explain it. If one considers the phosphorous ion as being effective, this is scarce in keeping with the toxic effects of 0.6% disodium acid phosphate where the percentages of sodium and phosphorus are much less and the pH about 8.4. With the disodium salt the tonicity of the solution may be a factor although twice the strength of that is isotonic with blood.

Calcium ions are apparently effective when their concentration is about 0.1 gram per 100 cc. or roughly ten times above the level for serum calcium. For magnesium there is a range of about 50 times the level of serum before any toxic effects are noted. Barium as might be expected is highly toxic. It may finally be added that the cercaricidal and lytic effects of rabbit's serum for the cercariae of *S. mansoni* could be demonstrated in vitro.

SUMMARY AND CONCLUSIONS

If one views the results obtained with various physical and chemical agents upon cercariae, one is surprised to find that so many of the cercariae to which man or experimental animal are exposed ultimately do develop and mature in the host. There certainly must be some mechanism for rapid adjustment to the tissues or fluids of the host once the cercariae have penetrated. For in vitro, there are primarily the cercaricidal and cercariolytic effects of serum; the restricting effects on mobility by the tonicity of the solutions, even if such movements have been greatly curtailed by the loss of the tail immediately on penetrating the skin; and the effects of body temperature which in itself representing as it does a rise of 10° C. or more above that of the water in the free living state is, in vitro, not without its injurious action upon cercariae. It is true that the tonicity of body fluids in itself is not detrimental to the parasite. In fact it is rather helpful inasmuch as it tends to restrict its activity; and as far as the body temperature is concerned it is conceivable that nutritive exchange with fluids of the host could compensate for the augmented expenditure of energy which cannot be balanced in the free living state of the cercariae. Such exchange of fluids through the cuticle of the developing schistosomule must occur until it reaches the stage of development where it is able to feed on the host's blood. In fact the effects of various salt solutions on the cercariae indicate that the cuticle behaves very much like a semipermeable membrane.

It is, however, admittedly difficult to explain how the cercariolytic effect of serum is counteracted.

From the practical standpoint perhaps the more important observation is the effect of sunlight and ultraviolet light on cercariae. Both are highly injurious under the described experimental conditions. Ultraviolet light from a mercury vapor lamp kills cercariae well within 45 minutes of exposure and strong sunlight within an hour. It is conceivable that under natural conditions, particularly in very shallow pools of water where liberated cercariae are close to the surface and where there is little or no foliage to offer protective shade, the direct effect of sunlight may play an important role in destroying them.

In conclusion, the following observations may be listed: (1) Cercariae can withstand refrigeration at 5° C. or 6° C. for from 4 to 6 days and remain infective when the temperature is again elevated. While many die off subsequently with longer refrigeration some may still remain alive for as long as 12 or 13 days. (2) The thermal death point for cercariae is about 6 hours at 40° C., 2 hours at 42° C., 1⅓ hours at 43° C. and ½ hour at 45° C. (3) Ultraviolet light permanently injures the cercariae after 20 minutes of exposure and is lethal within 45 minutes. Strong sunlight has almost the same effect. (4) Cercariae are unaffected by ranges of pH between 4.6 and 11.0. (5) Sodium chloride solution in the strength that it is present in blood plasma causes cercariae to permanently drop towards the bottom of the container, where they remain quite active and to a very limited degree infective for a mammalian host. 2.7% glucose solution has the same effect. (6) Potassium salts are toxic for cercariae when the concentration of K rises to about 0.1 gram per 100 cc. (7) Calcium is toxic for cercariae when its concentration reaches about 0.1 gram per 100 cc.

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