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Resistance of Pathogenic *Naegleria* to Some Common Physical and Chemical Agents

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Resistance of pathogenic *Naegleria* to drying, low and high temperature, and two halogens was studied. Drying made trophozoites nonviable instantaneously and cysts nonviable in <5 min. Trophozoites degenerated in hours at temperatures below 10°C and in minutes when frozen; cysts survived according to the equation $t_h = t_0/\theta = 1{,}440/1.122^T$ (t_0 is survival at 0°C; T is temperature between 0 and 10°C), but 1.5 h at −10°C to 1 h at −30°C. At 51, 55, 58, 63, and 65°C, trophozoites survived about 30, 10, 5, 1, and <0.5 min, respectively; cysts survived three to four times longer at 51°C and six to seven times longer at 55 to 65°C. Cyst destruction rates by heat indicated first-order kinetics with 25,400 cal/1°C for energy of activation. Cyst destruction rates by free chlorine and I_2 also conformed to first-order kinetics. Concentration-contact time curves yielded concentration coefficient values of 1.05 for free chlorine and 1.4 for I_2 and point to superchlorination as an effective means of destroying the cysts if free residuals are used as a guide and allowance is provided for low temperature and/or high pH waters.

The extensive presence of pathogenic *Naegleria* in the Orlando, Florida, lakes during the warm months (23), when viewed against a background of the occurrence of only five known cases of primary amoebic meningoencephalitis (PAM) that were associated with swimming in these lakes during the 12-year period between 1962 (2) and 1974 (S. L. Chang, 3rd Int. Congr. Parasitol., Munich, West Germany, 1974), points to the existence of unknown factor(s) related to the causative agent, the water environment, and the infective process and to the need of research in both the epidemiology and pathogenesis of the disease.

Meanwhile, it is essential to have a working knowledge of the resistance of this amoeba to some of the common physical and chemical agents. This is needed in understanding the mode of transmission and in managing the water environment, especially swimming pools, because pools fed with chlorinated river waters have been involved in the occurrence of PAM cases (1, 6).

MATERIALS AND METHODS

Pathogenic *Naegleria*. Strains from four geographic regions were used as representatives of the pathogenic *Naegleria*. The human case origins and culture histories of HB1 (Florida, 1968), TY (Virginia, 1969), HB3 (UTSi, Czechoslovakia, 1968), A1 (Australia, 1966), and GJ (Florida, 1973) strains have been described elsewhere (14; Chang, 3rd Int. Congr. Parasitol., Munich, West Germany, 1974). The GJ strain was included in the study because of its more recent isolation and its being somewhat more pathogenic and cytopathic than any of the other strains (Chang, 3rd Int. Congr. Parasitol., Munich, West Germany, 1974). All strains were carried in primary monkey kidney cell cultures and axenically in a calf serum-casein-glucose-yeast extract (CSCGYE) medium.

Trophozoites harvested from cell cultures were used in resistance tests to avoid the presence of cysts that might confuse the results.

To produce cysts, each strain was transplanted in monoxenic cultures on nonnutrient agar covered with a thin layer of heat-killed *Enterobacter aerogenes*. A fair to good crop of cysts was formed, usually in about 2 weeks of incubation at 35°C. Each plate was then washed with 5 ml of sterile distilled water, and the washings were pooled and centrifuged at 1,000 rpm for 20 min. The sediments were resuspended in enough distilled water to give the desired cyst concentration and were stored at 4 to 6°C until used. For use in testing cyst resistance to chemical agents, the final suspension was washed twice with, and resuspended in, demand-free water (21) to minimize the difference between the initial and residual concentrations of the agents. Cyst or trophozoite concentrations were determined by direct microscopic counting of the organisms in a drop of suspension and multiplying the count by the number of drops in 1 ml.

Primary monkey kidney cell cultures. The primary monkey kidney cell cultures were purchased from a commercial source. The growth medium consisted of medium 199 plus 0.5% albumin hydrolysate and 5% fetal calf serum. A concentration of 50 ng/ml each of penicillin and streptomycin or tetracycline was added to the medium before dispensing. All tube cell cultures were incubated at 35°C in a stationary phase.

CSCGYE medium. This medium, prepared earlier for axenic cultivation of pathogenic *Naegleria* (Chang,

3rd Int. Congr. of Parasitol., Munich, West Germany, 1974), consisted of an autoclaved base fluid, fetal calf serum, and fresh yeast extract. The base fluid contained (in percent, wt/vol) 0.1, isoelectric casein; 0.25, glucose; 0.15, Na_2HPO_4 and KH_2PO_4 in distilled water. To 88 parts of the base fluid were added 10 parts fetal calf serum and 2 parts fresh yeast extract (commercially available as a 25% solution). The medium was dispensed in 4- to 5-ml amounts in sterile, screw-capped culture tubes (15 by 150 mm), with or without antibiotics. Seeded tubes of CSCGYE medium were incubated in the same manner as tube cell cultures.

Effect of drying. In testing the resistance of pathogenic *Naegleria* to drying, 2 drops of a suspension containing about 1,000 trophozoites or cysts were spread over an area approximately 1 cm in diameter on each of a number of Leighton tube-adapted slides placed in a convenient number of sterile petri dishes. As soon as the visible fluid disappeared from a slide, it was immediately placed in a Leighton tube containing CSCGYE medium. The remaining slides were processed in the same manner at scheduled periods of time. A few slides were rehydrated with distilled water after 5-min, 1-h, and overnight drying and examined microscopically for morphological changes. All seeded Leighton tubes were incubated at 35°C and examined for amoebic growth up to 10 days.

Effect of heat. To ascertain the resistance of the amoebae to heat, tubes of the CSCGYE medium were placed in water baths at preset temperatures. After 30 min, 1 drop of a suspension containing about 1,000 trophozoites or cysts was placed in each tube and dispersed quickly by whirling the tube in the bath. After scheduled exposure times, tubes were removed in pairs and whirled in an ice-water bath to quickly reduce the temperature to 25 to 25°C. The cooled tubes were incubated and examined for amoebic growth as described above.

Having found that the cysts were considerably more resistant to heat than the trophozoites, as will be described later, we determined the rates of cyst destruction at different temperatures by the plaque count technique described in an earlier report (13).

A 1-ml amount of suspension containing 50,000 cysts was added to each test tube containing 9 ml of sterile distilled water. The tubes were prewarmed in water baths at preset temperatures. After scheduled exposure times, 1-ml samples were transferred into prechilled (iced) tubes containing 1 ml of sterile distilled water. The diluted samples and further fourfold dilutions thereof were plaqued in duplicate. After a 5- to 7-day incubation at 35°C, the plaques were enumerated. A control was prepared in the same manner but stored at room temperature, and its plaque count was used as the initial cyst concentration for computing the percent survival of cysts in the tests.

Effect of low temperatures. Low-temperature survival of pathogenic *Naegleria* was ascertained only in the cyst stage because preliminary tests showed that the trophozoites either degenerated or encysted when stored at temperatures between 12 and 20°C. They degenerated within hours at temperatures below 10°C and within minutes when frozen.

Prechilled tubes of the CSCGYE medium were seeded with about 1,000 cysts per tube and stored in a refrigerator at 4 to 5°C and in refrigerated water baths at 0 to 1, 8 to 9, and 14 to 15°C. After intervals of 1 to 5 days, tubes were removed in pairs, incubated, and examined for amoebic growth. In tests made at freezing temperatures, the seeded tubes were quickly frozen in a carbon dioxide-alcohol bath and then placed in freezers at −10 and −30° C. At 0.5-h intervals, tubes were removed in pairs, quickly thawed in a water bath at 35°C, and incubated for amoebic growth. To find out whether the serum and other organic matter in the medium had any protective effect on cyst survival under freezing, a suitable number of tubes were prepared with cysts suspended in distilled water and, after thawing, were centrifuged, and the water was replaced in the CSCGYE medium before incubation.

Effect of disinfectants. The effects of free chlorine ($HOCl\text{-}OCl^-$) and elemental iodine (I_2) were examined because they are commonly used in water disinfection. All tests were made in demand-free water at 25°C; with free chlorine, the pH was 7.2 to 7.3, and with I_2, it was 6.0.

The test procedure differed from that employed in determining cyst destruction rates by heat in the following manner: (i) the cyst-laden water was dispensed in 200-ml amounts in 300-ml Erlenmeyer flasks; (ii) a halogen neutralizer containing 0.01 N each of Na_2SO_3, and $Na_4P_2O_7$ and 0.1% glycerine (15) was used to terminate each contact time; (iii) a cyst concentration of 2,500 cysts per ml was used; (iv) 10-ml samples were taken at scheduled contact times and blown into tubes, each containing 0.5 ml of the neutralizer; and (v) all neutralized samples were plaques in triplicate, both undiluted and in fourfold dilutions, to ascertain the cyst survival.

RESULTS AND DISCUSSION

Resistance to physical agents. (i) Drying. Results obtained in three replicate tests made with the GJ, TY, and A1 strains of pathogenic *Naegleria* in drying in air at 26°C and 22% relative humidity are shown in Table 1.

Table 1 shows the rapidly lethal effect of drying on pathogenic *Naegleria,* especially in the tropic stage. Microscopic examinations of the rehydrated slides revealed loss of refractility and granular cytoplasm of trophozoites as soon as the visible fluid disappeared from the slides and of cysts after 5 min of drying. Space sepa-

TABLE 1. *Resistance of pathogenic Naegleria to drying in air at 26°C and 22% relative humidity*

Stage of amoeba	Period of drying	No. of tests showing positive cultures for strain:		
		GJ	TY	A1
Trophozoites	0[a]	0/3	0/3	0/3
	5 min to 24 h	0/3	0/3	0/3
Cysts	0[a]	3/3	2/3	3/3
	5 min to 24 h	0/3	0/3	0/3

[a] Placed in CSCGYE medium immediately after the disappearance of visible fluid from slides.

rating the cyst wall and shrunken cytoplasm were noticed after longer periods of drying. It appears that the lethal effect of drying is attributable to the loss of cytoplasmic water, thus causing denaturation of proteins. The slightly longer survival of cysts was probably due to the somewhat greater resistance to such water loss of the cyst wall than that of the cytoplasmic membrane. The slightly inconsistent results on cyst survival after zero period of drying indicates the lack of precision in visual judgment of the state of dryness.

Worth noting is that these results were similar to those obtained in an early study made on the cysts of *Endamoeba histolytica* (7) but were in sharp contrast to the at least 23-month survival of the *Naegleria gruberi* cysts on similarly dried slides (10). Similar cytoplasmic changes were exhibited by the *E. histolytica* (7), but the *N. gruberi* cysts retained their normal appearance upon rehydration after 23 months of drying (10). Apparently, the latter amoeba can retain its cytoplasmic integrity after losing its water and, hence, preserve its survival.

The failure to isolate pathogenic *Naegleria* from the swimming pool that was associated with the cases of PAM in Czechoslovakia (4) and the isolation of *Acanthamoeba* from the floor dust in the barracks as well as from the nasal swabs of the occupants led to the belief that the *Naegleria*-caused PAM may also result from inhalation of the dust-borne cysts (5). This belief, however, is not supported by the present data. Carter (3) also reported that cysts of the pathogenic *Naegleria* quickly became nonviable when dried and dismissed the hypothesis of inhalation of air-borne cysts as a means of infection.

(ii) Low temperature. Data obtained in tests made with cysts of the GJ, TY, and A1 strains on storage at temperatures ranging from −30 to 14 to 15°C are shown graphically in Fig. 1, with the points representing midpoints of storage time between the last positive and first negative cultures of each strain. The figure shows an inverse, linear relationship between the log survival time and the temperature between 0 and 1 and 14 to 15°C, which can be quantitatively expressed by an empirical equation employed earlier (7).

$$t_h = \frac{t_0}{\theta} = \frac{1{,}440}{1.122^T} \qquad (1)$$

where t_h is the survival time in hours, t_0 is the survival time at 0°C (without freezing), T is the storage temperature in degrees centigrade, and θ is the temperature coefficient. Because the cysts may excyst at temperatures of and above

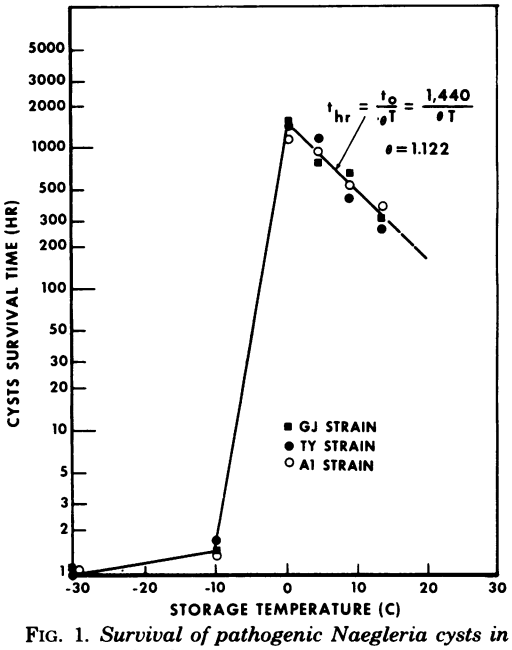

FIG. 1. *Survival of pathogenic Naegleria cysts in water at varying low temperatures.*

20°C, the equation holds at values for T between 0 and 19°C.

It should be noted that the value of t_0 is affected by the cyst concentration employed. For instance, plate cultures were found positive upon transfer up to 2.5 months of storage at 4 to 5°C. The some 2-weeks-longer survival can be attributed to the 100- to 200-times-greater cyst population in the cultures than that in the test suspension.

The detrimental effect of freezing on cysts was demonstrated by the sudden, sharp shift from long survival above, to a much shortened survival below freezing. No significant difference was observed in survival time between cysts suspended in the CSCGYE medium and those in water. The 1-h survival at −30°C to 1.5-h survival at −10°C indicates that the quicker the cysts become frozen, the faster they become nonviable.

Microscopic examinations of cysts thawed within the survival period revealed normal appearance in high percentages at the beginning of the survival time to low percentages toward the end. Cysts thawed after the survival time showed cytological changes similar to those observed in drying. It appears, hence, that the lethal effect of freezing is a result of crystallization of cytoplasmic water, thus causing denaturation of proteins similar to the loss of water in drying.

In this connection, it is interesting to note that

in an early study on survival of *E. histolytica* cysts suspended in various fluids, the t_{day} was found equal to $87.21/1.119^T$ (7), and in another study on cysts of *E. histolytica* in feces (9), the t_h was found equal to $1,500/1.13^T$. Of particular interest is that the value of 87.2 days for t_0 was obtained with cyst concentrations 50 to 100 times greater than that used in the present study, whereas the value of 1,500 h for t_0 was obtained with a cyst concentration comparable to that used in the present study. They further substantiate the belief that, with other factors constant, the survival time is influenced by the concentration of cysts used.

Also worth noting is that the very short survival of the pathogenic *Naegleria* cysts in freezing is shared by the cysts of *E. histolytica,* either suspended in water (7) or mixed in feces (9); it is, however, in sharp contrast to the 3.5- to 4-month survival of the *N. gruberi* cysts stored at −25 to −30°C (10). Because the latter cysts also showed no cytoplasmic changes during the 4-month freezing (10), it is apparent that the long survival should also be attributed to the ability of the cysts to retain their cytoplasmic integrity after crystallization of the water, as was the case in drying. In this connection, it is worth noting that cysts of both the pathogenic *Naegleria* and *E. histolytica* were rendered nonviable by lyophilization, whereas those of the *N. gruberi* remained viable after at least 6 months in a lyophilized state (Chang, unpublished data).

In a practical sense, these observations imply that the pathogenic *Naegleria* is unlikely to establish permanent growth in fresh surface waters in regions where the winter is too cold and extends beyond the maximal survival period of the cysts. This may well explain the isolation of pathogenic *Naegleria* in Belgium in the winter only in a thermally polluted stream (19) and warm industrial wastes (16) and in continual isolation throughout the year from all but one of the Orlando lakes, the water temperature of which dropped to between 12 and 18°C only in the months of January and February (23).

(ii) High temperature. The growth of pathogenic *Naegleria* in cultures at 43 to 45°C has been reported (14, 20) and employed in its isolation in the field (23). The amoeba, however, can tolerate much higher temperatures in short exposures. This is demonstrated in Table 2, which shows the data obtained in the semiquantitative tests made with the GJ, HB1, HB3, and A1 strains suspended in the CSCGYE medium and exposed to the temperatures in the 51 to 65°C range.

In Table 2, it is seen that the trophozoites survived on the average 30, 10, 5, 1, and <0.5 min, and cysts survived 120, 60, 30, 7, and 2.5 min at 51, 55, 58, 63, and 65°C, respectively. It appears that the cysts are three to four times as resistant as the trophozoites at 51°C and six to seven times as resistant at 55 to 65°C.

The exposure time-temperature combination—the so-called thermal death point—is, however, not a constant but changes with the change in organism concentration employed. As will be seen later, thermal destruction of unicellular organisms is, kinetically, a rate process; hence, the higher the concentration of organisms in the destructive process, the longer the exposure time required to reach a fixed percentage of destruction, with other variables remaining constant. Under the present testing conditions, the maximal survival time implied a cyst destruction close to 99.95%, assuming that every cyst was a surviving unit. If a higher cyst concentration were used, the exposure time to reduce the cysts to a single survivor per tube would have to be proportionally longer.

Data from the quantitative tests on rates of

TABLE 2. *Relative resistance of high temperature of the trophozoites and cysts of pathogenic Naegleria suspended in the CSCGYE medium*

Strain	Stage of amoeba	Survival time (min)[a] at stated temp (°C)						
		51	52	53	55	58	63	65
GJ	Trophic	45	40	30	15	7.5	1.5	<0.5
	Cyst	145	ND[b]	ND	75	45	9	2.5
HB1	Trophic	35	35	35	10	4.5	1	<0.5
	Cyst	130	ND	ND	60	35	7	3
HB3	Trophic	25	25	20	7.5	5.5	1	<0.5
	Cyst	105	ND	ND	45	25	7	2.5
A1	Trophic	35	40	30	10	5.5	0.5	<0.5
	Cyst	120	ND	ND	60	35	5	2

[a] Midpoint-point of exposure time between last positive and first negative cultures.
[b] ND, Not done.

destruction of cysts of the HB1, HB3, and A1 strains at 55, 57, 60, and 65°C are shown graphically in Fig. 2, with each point representing the average of two replicate plaque counts of each strain. Figure 2 demonstrates an exponential survival of the cysts at all four temperatures and a comparable heat tolerance of cysts among the three strains.

The relationship between the destruction rates and the temperatures exposed has been commonly expressed in reference books on physical chemistry by the following equation:

$$\log \frac{k_2}{k_1} = \frac{E}{2.3R} \frac{T_2 - T_1}{T_1 T_2} \quad (2)$$

where k_1 and k_2 are the reaction or destruction rate constants at the absolute temperatures T_1 and T_2, respectively, E is the energy of activation in the destructive process, and R is the gas constant (1.99 cal/1°C). Equation 2 can be written as:

$$\log \frac{k_2}{k_1} \frac{T_2 T_1}{T_2 - T_1} = \frac{E}{2.3R} \quad (3)$$

Hence, when the values for k are plotted on a log scale against the reciprocals of the respective values for T, the value for E can be computed directly from the slope, which is the left side of equation 3, as shown in the following expression:

$$\text{Slope} = \frac{E}{2.3R} \quad (4)$$

Such a plot is shown in Fig. 3. From the slope, the value for E was computed to be 25,400 cal/1°C.

The relatively large value for the energy of activation in the thermal destruction of the pathogenic *Naegleria* cysts indicates that the destructive process is compatible to that in denaturation of proteins.

For practical purpose, the Q_{10} commonly used to determine the effect of 10-degree change in temperature on destruction or reaction rate is more useful than the E. It is computed by the equation:

$$Q_{10} = \left(\frac{k_2}{k_1}\right)^{\frac{10}{T_2 - T_1}} \quad (5)$$

Since in exponential survival the time, t, for a fixed percent destruction is reciprocal to k, equation 5 can also be written as,

$$Q_{10} = \left(\frac{t_1}{t_2}\right)^{\frac{10}{T_2 - T_1}} \quad (6)$$

If T_1 and T_2 are selected so that T_2 is 10 degrees higher than T_1, the value for Q_{10} can be computed from k_2/k_1 or t_1/t_2.

With the values for k obtained from the survival curves in Fig. 2, the value for Q_{10} was computed to be very close to 2. The *E. histoly-*

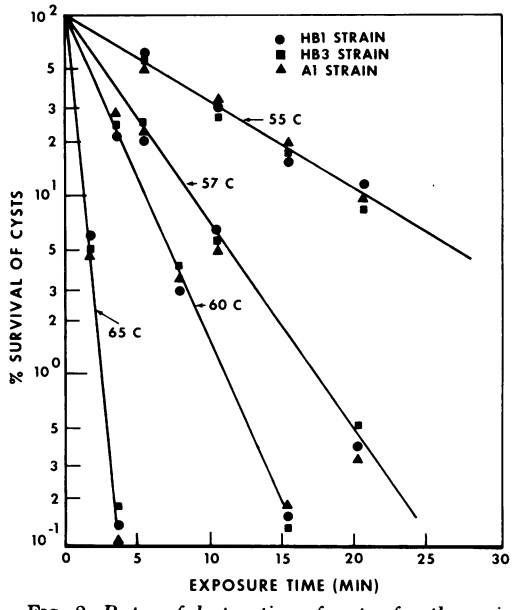

FIG. 2. *Rates of destruction of cysts of pathogenic Naegleria in water by heat.*

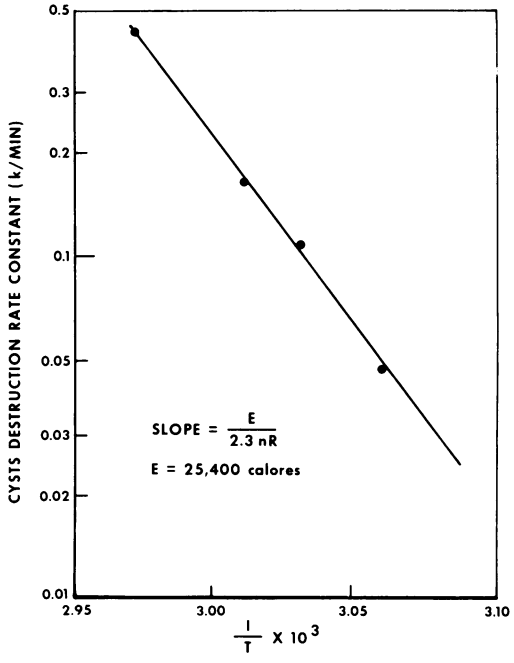

FIG. 3. *Energy of activation (E) in the destruction of pathogenic Naegleria cysts by heat.*

tica survival data yielded a Q_{10} value of 7.5 (8). They imply that at a 10-degree increase in temperature above the physiological range, the thermodestruction of pathogenic *Naegleria* cysts is doubled and that of *E. histolytica* cysts is increased 7.5 times.

To see how these data compare with those obtained in the semiquantitative tests, the exposure times for a 99.9% cyst destruction were extrapolated from the survival curves in Fig. 2 and came out as 65, 26, 17, and 3 min at 55, 57, 60, and 65°C, respectively. These values are in reasonable agreement with those shown in Table 2, taking into consideration the slight differences in three of the four temperatures used in these two groups of tests.

In this connection, it is of interest to note that the midpoints of exposure times between the last positive and first negative cultures in the thermo destruction of the *E. histolytica* cysts were 115, 30, 7.5, and 2 min at 45, 47, 49, and 50°C, respectively, and the value for E was 134,000 cal/1°C (8); cysts of the *N. gruberi* were destroyed in 32.5 min at 50°C and 1.5 min at 60°C (10). Because these early data were obtained with cyst concentrations comparable to that used in the present study, it is apparent that the cysts of pathogenic *Naegleria* are much more resistant to heat than those of either of the other two species of amoebae and that the *E. histolytica* cysts are somewhat more sensitive to heat than those of the *N. gruberi*.

Resistance to chemical agents. Data on the destruction of cysts of the HB1, TY, and A1 strains by free chlorine at pH 7.2 to 7.3 and by I_2 at pH 6.0 in water at 25°C are graphed in Fig. 4, with each point representing the mean of three replicate plaque counts of each strain. The survival curves in the figure clearly demonstrate a first order reaction in the destructive process by either halogen compound and also a comparable resistance among these strains.

Of special interest is that the cyst destruction rates by the two halogen compounds were quite similar at the 3.1 to 3.4 ppm level, above which I_2 became increasingly more cysticidal than free chlorine and below which the relationship was reversed. The phenomenon indicates that the two halogen compounds have clearly different concentration coefficient in their cysticidal activity. To ascertain this difference quantitatively, the van't Hoff (22) equation is employed, as follows:

$$K = C^n t \tag{7}$$

where K is a constant, C is the concentration of halogen, t is the contact time for a fixed percentage of cyst destruction, and n is the halogen

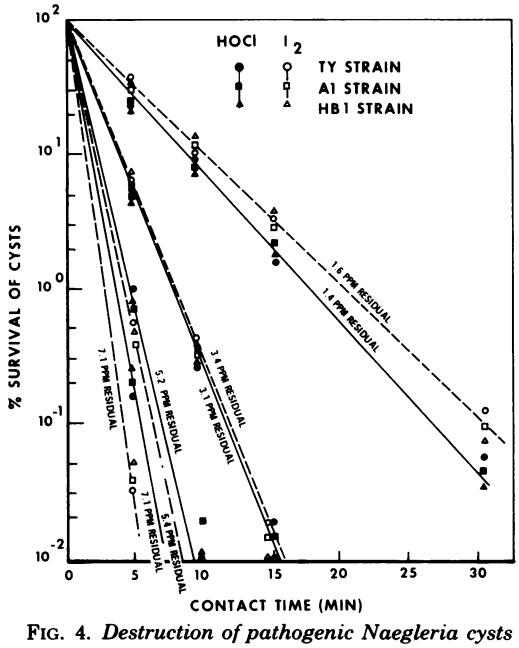

FIG. 4. *Destruction of pathogenic Naegleria cysts in water at 25°C by free chlorine at pH 7.2 to 7.3 and by I_2 at pH 6.0.*

concentration coefficient. Equation 7 can be expanded to:

$$K = C_1^n t_1 = C_2^n t_2 \ldots \tag{8}$$

Hence,

$$n = \frac{\log(C_2/C_1)}{\log(t_1/t_2)} \tag{9}$$

Equation 9 dictates a linear relationship between the log C and log t, the slope of which is the value for n. With the values for t extrapolated from the respective survival curves in Fig. 4 at the 99.9% destruction level and plotted against the respective halogen residuals on a log-log scale in Fig. 5, two linear curves are produced—the one for the free chlorine being slightly steeper that that for I_2. By employing equation 9, the values for n were computed as 1.05 for the former and 1.4 for the latter. They imply that doubling the concentration of free chlorine cuts the contact time for a fixed percent of cyst destruction by one-half; doubling the concentration of I_2 cuts the contact time by about two-thirds.

It is interesting to note that in the destruction of cysts of *E. histolytica* in water, the values for n were 1.0 for hypochlorous acid (free chlorine at pH 6.0) (19) and 1.4 for I_2 (11). Also of interest is that in comparing these two sets of concentration-contact time curves, no significant differ-

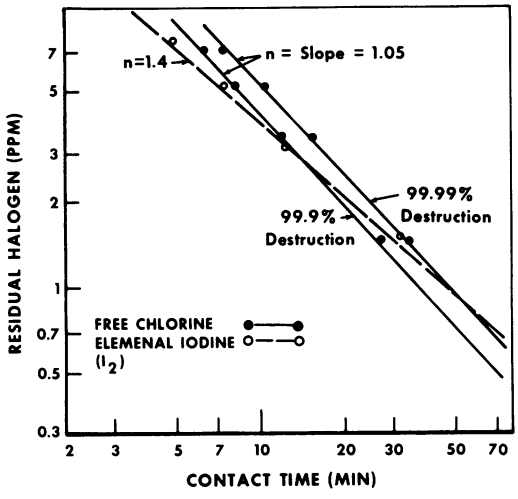

FIG. 5. *Concentration-contact time relationship in the destruction of pathogenic Naegleria cysts in water at 25°C by free chlorine at pH 7.2 to 7.3 and by I_2 at pH 6.0.*

ence to either halogen was found between these two entirely different species of amoebae.

In another early report, it was shown that the midpoints of contact times between the last positive and first negative cultures in the destruction of cysts of *N. gruberi* in water at 26 to 27°C were 32.5 and 12.5 min at 1.5 and 3.5 ppm residual free chlorine or I_2, respectively (10). These values were very close to those predicted by the respectives curves in Fig. 5, indicating cysts of these two more closely related species also have similar resistance to both halogens.

The term *n* has also been considered to indicate the order of reaction by van't Hoff (22). In such consideration, the value of 1.0 or 1.05 for *n* is in agreement with the first-order reaction observed for free chlorine, but the value of 1.4 for *n* would suggest a one and one-half-order reaction for I_2, which is not in agreement with the exponential curves. In another study made on the *N. gruberi* cysts, using $^{131}I_2$ as a tracer, it was calculated that 1.2×10^6 molecules of I_2 were required to destroy a single cyst (12). It appears, then, that whereas the cyst destruction data support a first-order kinetics, the reaction is actually a pseudo-first-order process. Insofar as it takes the same number of molecules of a compound to effect a "kill" of discrete, single-cell organisms, there will be a fixed percent survival per unit of contact time, and the process resembles that of the first-order reaction (12).

In their tests made with free chlorine on cysts of the HB1 strain in water at 25°C and pH 7.3 to 7.4, de Jonckheere et al. (18) obtained first negative cultures with residuals of 0.3, 0.8, and 2.0 ppm in 1, 0.4, and 0.4 h, respectively, and on cysts of *N. gruberi* obtained cultures with residuals of 0.1, 0.6, and 2.0 ppm in 3, 1, and 0.5 h, respectively. These authors used a cyst concentration of 1,000/ml and a membrane filter concentrate of 10 ml per inoculum; their first negative cultures, therefore, implied a cyst destruction slightly greater than 99.99%, assuming again that every cyst was a surviving unit.

To facilitate a fair comparison between the data of these authors and those obtained in the present study, a 99.99% destruction curve was extrapolated from the survival curves in Fig. 4 and is plotted in Fig. 5. In fitting the data points of these authors to the curve, it was found that whereas the 0.8-ppm-in-1-h point fell almost on the curve, the 0.3- and 2.0-ppm residuals had their contact times about one-half of what the curve indicated. Since these authors used 1-, 3-, and 24-h contacts after the 30-min contact, it is likely they had missed the most probable last positive cultures somewhere between these contact times.

De Jonckheere et al. concluded that the cysts of *N. gruberi* are somewhat more resistant to free chlorine than those of the pathogenic *Naegleria* and felt that chlorination is an effective method for destroying the cysts in swimming pools and domestic supplies because short superchlorination up to 10 ppm is in common practice in water-supply plants using surface water in Belgium. On the other hand, failure to eradicate the *Naegleria* amoeba has been reported with 10 ppm of chlorine in a backyard swimming pool that was filled with a domestic supply of chlorinated river water and responsible for the occurrence of cases of PAM in South Australia (1).

From the data shown in Fig. 5, it appears that a satisfactory destruction of the *Naegleria* cysts in water is within the range of superchlorination as de Jonckheere et al. had stated, but the effectiveness must be judged not by the initial but by the residual free chlorine with the available contact time. Allowance of free chlorine must also be provided to compensate for lower temperature and/or higher pH of the water to be treated. In the case of swimming pool disinfection, cyst destruction in the pool system must be done before the pool is in use because the residual free chlorine in the practical range and the uncertainty of the contact time make it difficult, if not impossible, to assure a satisfactory cyst destruction.

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LITERATURE CITED

1. **Anderson, K., and A. Jamieson.** 1972. Primary amoebic meningo-encephalitis. Lancet **i**:902-903.
2. **Butt, C. G.** 1966. Primary amoebic meningoencephalitis. N. Engl. J. Med. **274**:1473-1476.
3. **Carter, F. R.** 1970. Description of a *Naegleria* sp. isolated from two cases of primary amoebic meningoencephalitis, and the experimental pathological changes induced by it. J. Pathol. **100**:217-244.
4. **Červa, L.** 1971. Studies on limax amoebae in a swimming pool. Hydrobiologia. **38**:141-161.
5. **Červa, L.** 1973. Isolation of limax amoebae from the nasal mucosa of man. Folia Parasitol. (Prague) **20**:97-103.
6. **Červa, L., K. Novak, and C. G. Culbertson.** 1968. An outbreak of acuate, fatal amoebic meningoencephalitis. Am. J. Epidemiol. **88**:436-444.
7. **Chang, S. L.** 1943. Studies on *Endamoeba histolytica*. II. Observations concerning encystation, maturation and excystation of *E. histolytica*, and on longevity of culture-induced cysts in various fluids at different temperatures. J. Infect. Dis. **72**:232-241.
8. **Chang, S. L.** 1952. Kinetics in the thermo-destruction of cysts of *Endamoeba histolytica* in water. Am. J. Hyg. **52**:82-90.
9. **Chang, S. L.** 1955. Survival of cysts of *Endamoeba histolytica* in human feces under low temperature conditions. Am. J. Hyg. **61**:103-120.
10. **Chang, S. L.** 1958. Cytological and ecological observations on the amoeba state of *Naegleria gruberi*. J. Gen. Microbiol. **18**:565-578.
11. **Chang, S. L.** 1958. Use of active iodine as a water disinfectant. J. Am. Pharm. Assoc., Sci. Ed. **47**:417-423.
12. **Chang, S. L.** 1971. Modern concept of disinfection. J. Sanit. Eng. Div. Am. Soc. Civ. Eng. **97**:417-423.
13. **Chang, S. L.** 1971. Small, free-living amoebae: cultivation, quantitation, identification, classification, pathogenesis, and resistance. Curr. Top. Comp. Pathobiol. **1**:201-254.
14. **Chang, S. L.** 1974. Etiological, pathological, epidemiological, and diagnostical considerations of primary amoebic meningoencephalitis. Crit. Rev. Microbiol. **3**:135-159.
15. **Chang, S. L., and G. Berg.** 1959. Chlormelamine and iodized chlormelamine formulations: essential physical and chemical properties and germicidal efficiencies. U.S. Armed Forces Med. J. **10**:33-49.
16. **De Jonckheere, J., and H. Van de Voorde.** 1976. Differences in destruction of cysts of pathogenic and nonpathogenic *Naegleria* and *Acanthamoeba* by chlorine. Appl. Environ. Microbiol. **31**:294-297.
17. **De Jonckheere, J., and H. Van de Voorde.** 1977. The distribution of *Naegleria fowleri* in man-made thermal waters. Am. J. Trop. Med. Hyg. **26**:10-15.
18. **De Jonckheere, J., P. Van Dijck, and H. Van de Voorde.** 1975. The effect of thermal pollution on the distribution of *Naegleria fowleri*. J. Hyg. Camb. **75**:7-13.
19. **Fair, G. M., J. C. Morris, and S. L. Chang.** 1947. Dynamics of water chlorination. J. N. Engl. Water Works Assoc. **61**:285-301.
20. **Griffin, J.** 1972. Temperature tolerance of pathogenic and nonpathogenic amoebas. Science **178**:869-870.
21. **Scarpino, V. P., Gerald Berg, S. L. Chang, et al.** 1972. A comparative study of the inactivation of viruses in water by chlorine. Water Res. **6**:959-965.
22. **Van't Hoff, J. H.** 1896. Studies in chemical dynamics, p. 19. Chemical Publishing Co., Easton, Pa.
23. **Wellings, F. M., A. L. Lewis, P. Y. Amus, and S. L. Chang.** 1977. *Naegleria* and water sports. Lancet **i**:199-200.