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Limax Amebae in Two Swimming Pools at Iowa State University, Ames, Iowa

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Four types of amebae (*Acanthamoeba, Hartmanella, Naegleria*-like amebae, and a vannellid, probably *Platyamoeba*) were identified from 2 indoor swimming pools on the Iowa State University campus during January-April 1981. Large numbers of these amebae, and other protozoans and metazoans were found in a yellow-brown (mainly bacterial) floc growing on the pool bottom, in filtering systems, and in recirculation pipes. None of the amebae were found to be pathogenic in laboratory mice. Corrective pool maintenance procedures and laboratory studies of the floc and amebae are described.
INDEX DESCRIPTORS: Limax amebae (*Naegleria*-like amebae, *Acanthamoeba, Hartmanella, Platyamoeba*), bacterial floc, swimming pools, Iowa State University.

Freeliving amebae are common in lentic freshwater environments and in moist soil. Some of these amebae may be expected to occur in biologic communities which develop on water filter surfaces. Limax amebae such as *Acanthamoeba, Hartmanella,* and *Naegleria* species are mainly coprophilous and may often be isolated from sources of high organic content. These 3 genera also contain species that may be facultative parasites of vertebrates; human infections have been associated with swimming in contaminated freshwater lakes or swimming pools (chiefly warm outdoor swimming pools).

Resistant cysts of these amebae may gain access to indoor and outdoor swimming pools from water supplies and swimmers. Although routine filtration and chlorine disinfection may be expected to eliminate these organisms, populations may thrive in wall cracks and crevices inaccessible to normal cleaning and disinfection (Kadlec *et al.*, 1978), or may be unaffected by low concentrations of disinfectants.

Two species, *Naegleria fowleri* Carter and *Acanthamoeba culbertsoni* (Singh and Das), are potential human pathogens which may cause primary amebic meningoencephalitis (PAM). The amebae are introduced when contaminated water enters the nasal passages; access to the brain is gained via the olfactory nerves. Brain tissue is destroyed rapidly, and PAM is highly lethal (approx. 90% fatal) in human beings, rats, mice, monkeys, and probably other mammals. PAM progresses rapidly to death, making diagnosis difficult. Certain anti-fungal agents such as amphotericin B administered in heavy doses and sulfa drugs (sulfadiazine) show therapeutic promise (Griffin, 1978). Cases of PAM have been reported from several European nations, Australia, New Zealand, and from Florida, Texas, Georgia, and Virginia (Griffin, 1978). Most cases have resulted from swimming in freshwater lakes; cases associated with swimming pools have occurred in Czechoslovakia, New Zealand, and Belgium. Pathogenic species of *Naegleria* and *Acanthamoeba* are known to be heat and chlorine resistant (Cerva, 1971; Griffin, 1972).

During January 1981 large populations of *Naegleria*-like amebae and 3 other genera of limax amebae were found growing in a yellow-brown floc on the bottom of the swimming pools and filter systems in Beyer Hall and in the Physical Education Building on the campus of Iowa State University (ISU).

DESCRIPTION OF POOLS AND MAINTENANCE

Beyer Hall Pool (BHP) and the Physical Education Building Pool (PEBP) contain volumes of 300,000 gallons and 90,000 gallons, respectively. The filtration system of BHP consists of two 8 ft. x 20 ft. horizontal pressure double-cell sand filters operating at 2.7 gal/ft^2/min. The recirculation rate is 860 gpm with the backwash rate 13 gal/min/ft^2. Turnover time for BHP is 5.8 h. Filtration of the PEBP is accomplished by 6 dual surface 2.5 ft. x 3 ft. diatomaceous earth vacuum filters with a filter rate of 2.7 gal/ft^2/min, and a recirculation rate of 240 gpm; turnover time for PEBP is 6.25 h. Disinfection in both pools during the amebae outbreak was by bromine compounds (Aquabrome-approximately 25% chlorine) in "stick" erosion feeders.

Prior to the outbreak, routine water analyses of both BHP and PEBP showed the following: pH 7.2-7.4, temperature 27°-29°C, bromine 0.5-0.8 mg/1, total alkalinity 80-90 mg/1. Prior to and during the outbreak, most probable number coliform bacterial analyses of pool water were negative; total plate counts of pool water were negligible.

SAMPLING METHODS AND SOURCE OF AMEBAE

Pool bottom samples (obtained by scuba diving) and filter samples were drawn intermittently from late December 1980 through May 1981. Amebae were examined in fresh smears prepared from undiluted pool water samples containing yellow-brown floc. To induce cyst formation, samples were incubated for 24-48 h at room temperature and at 10°C on plates of non-nutrient agar covered with distilled water. Samples of yellow-brown floc in pool water, in pool water diluted 1:1 with distilled water, and on non-nutrient agar plates were observed for up to 30 days.

During December 1980, yellow-brown floc (Fig. 1) was noted in the main drain lines, in the surge tank and on the filter sand in BHP. Small amounts of this material were also seen on the bottom of BHP. In an attempt to eliminate the floc, the pool was superchlorinated (18 mg/1) by High Test Hypochlorite (HTH) powder and liquid bleach in late December. Yellow-brown material persisted on the pool bottom and in the surge tank following this disinfection. It was thought that growth of the yellow-brown floc in BHP may have resulted from a breakdown in one sand filter and consequent overloading of the second filter for approximately 3 months (October-December 1980). In early January 1981 the same yellowish material appeared in the PEBP filter tank. Except for superchlorination in BHP, the pools had minimal maintenance during a partial shutdown of the ISU campus to conserve energy during the 1980 Christmas-New Year holiday. Ambient temperature in Beyer Hall and in the Physical Education Building during the holiday break was reduced to 12.7°C. Lack of enforcement of a showering requirement for swimmers may also have contributed to the pool contamination.

Samples of the yellow-brown floc were examined microscopically at ISU, the Iowa State Hygienic Laboratory, and the Center for Disease Control (CDC). The bulk of the yellow-brown material was composed of bacterial colonies supporting growth of large numbers of protozoan and metazoan organisms including amebae, phyto- and zooflagellates, ciliates, rotifers, gastrotrichs, and free-living nematodes and nematode eggs. Some samples showed fungal or green algal filaments (Fig. 2), but these were not definitely

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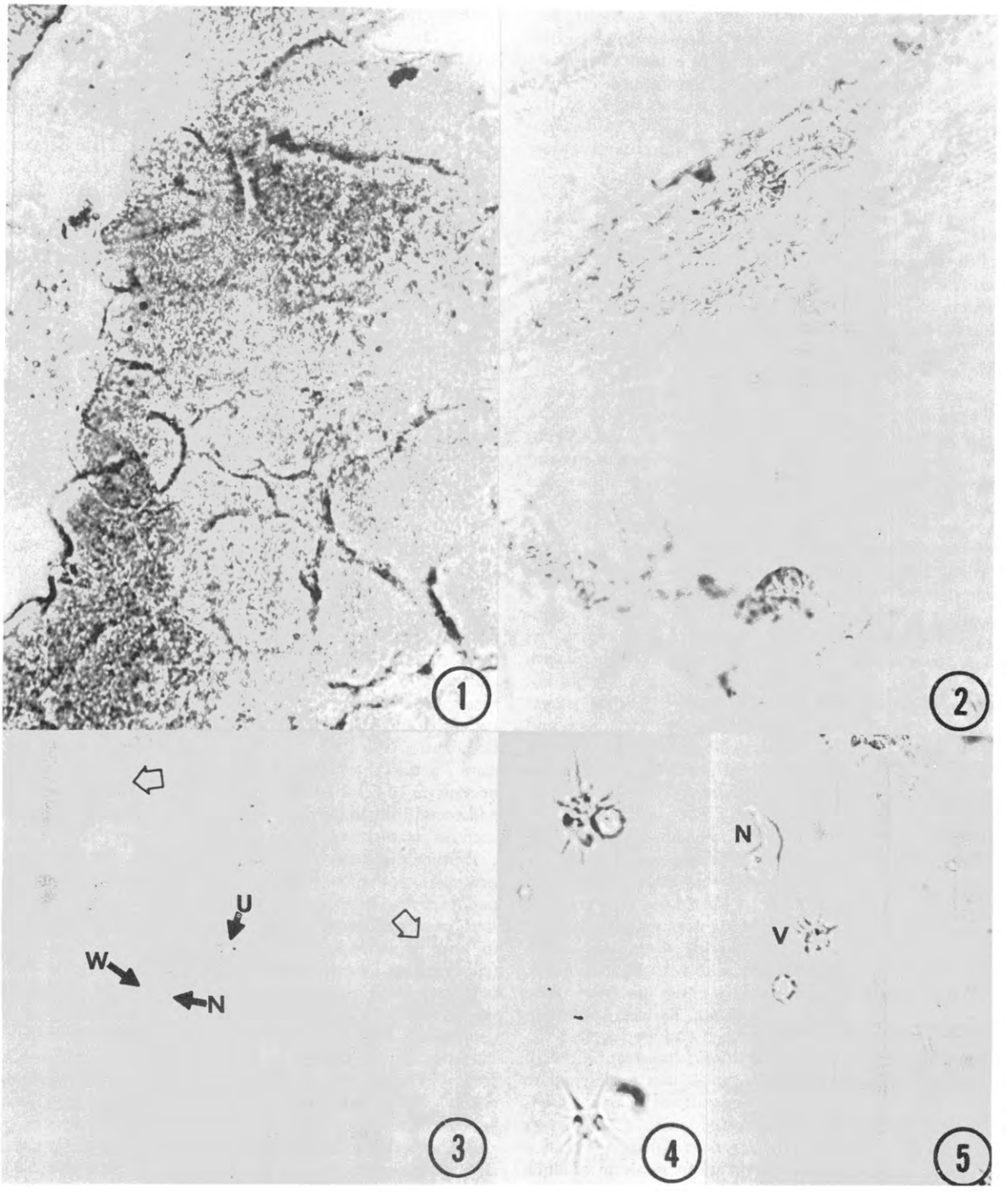

Fig. 1. Dense matrix of yellow-brown bacterial floc from swimming pools. Approx. 100X. Fig. 2. Loose filamentous components of floc. Approx. 500X. Fig. 3. Motile *Naegleria*-like ameba showing anterior eruptive wave (W), nucleus (N), and posterior uroid (U). Arrows indicate bacterial floc. Approx. 500X. Fig. 4. Vannellid ameba, floating radiate stages. Approx. 500X.Fig. 5. Stellate vannellid (V) and *Naegleria*-like (N) amebae. Approx. 350X.

identified. Organic debris including human hair and other detritus was also common in the heavy bacterial growth.

Qualitative chemical analyses (x-ray diffraction analysis) performed by the Materials Analysis Laboratory, Engineering Research Institute at ISU showed the yellow-brown floc to contain the following elements in decreasing order of relative concentrations: C, P, K, Fe, Mg, S, Cl, Al, Si, Na, Br. Preliminary studies indicated the bacteria composing the yellow-brown floc were species of *Bacillus, Pseudomonas* and *Alcaligenes*. The predominant species was a slow growing spore forming *Bacillus*.

IDENTIFICATION OF AMEBAE

Four types of amebae were recognized in the yellow-brown floc samples taken from both pools during January and February 1981. *Naegleria*-like vahlkamfiid amebae (Fig. 3) were seen in both trophic and cystic stages. For several minutes after slide preparation the trophic stage of these amebae remained rounded and inactive; first signs of activity were broad pseudopodia projecting from 1 or 2 surfaces. Once active, the trophic amebae were elongate, showed a distinct nucleus with a prominent endosome, measured 5-15 x 1.5-4 μm and moved rapidly by clear, nongranular eruptive waves. A prominent club-shaped uroid was evident on all active specimens and usually appeared covered with fine protoplasmic projections resembling rod-shaped components of the yellow-brown floc (Fig. 3). While moving across a microscopic field, several specimens "cast off" the protoplasmic projections from the uroid. Pigment in food vacuoles in these amebae was probably derived from the yellow-brown swimming pool floc.

Naegleria-like cysts appeared in pool water samples and on non-nutrient agar plates held for 24 h at room temperature and at 10°C. Cysts were spherical, 6-16 μm (mean of 10 = 10μm) in diameter with a single central nucleus; pores were seen in some cyst walls, but not in others. Transformation to the transient flagellate stages characteristic of *Naegleria* was not observed; however, many bi- and quadriflagellate forms closely resembling literature descriptions of *Naegleria* were seen in one PEBP sample diluted 1:1 with distilled water and incubated 24 h at 10°C. No active ameboid stages were seen in this sample.

Trophic stages of another ameba, a member of the family Vannellidae (probably *Platyamoeba*), were also common in freshly examined samples from both pools; spherical cyst-like bodies, possibly of this species, were very common in 24-48 h old samples, but en- or excystation was not observed. Vannellid amebae were stellate or radiate (Figs. 4, 5) in the floating stage; flattened nonradiate forms preceding and following the radiate stages were virtually motionless, slightly longer than broad, with a clear crescentic "anterior" zone and a globular vesicular "posterior" region. Radiate specimens showed clear, conical, sometimes branched, pseudopodia.

Dr. G. Visvesvara isolated and identified representatives of *Platyamoeba, Acanthamoeba* and *Hartmanella* in samples sent to the CDC in January 1981. *Naegleria* was not identified in the CDC samples. Several mycetozoans form limax amebae and show flagellate stages remarkably similar to *Naegleria* (Olive, 1975). It is possible at least some of the *Naegleria*-like amebae were of slime mold origin, although mycetozoans were not found in floc samples.

LABORATORY STUDIES

Pathogenicity tests were performed in white laboratory mice. A preliminary test was carried out on 9 January 1981 at ISU using the initial pool samples. Intranasal doses of approximately 10^4 trophic and encysted amebae taken directly from pool samples were administered to 2 mice. Neither animal developed any signs of disease over a 3-week period of observation. Mouse tests performed at the CDC in February were also negative. Suitable numbers of amebae for the CDC tests were cultured from pool samples taken in early January. In CDC tests for temperature tolerance, only *Hartmanella* amebae appeared in cultures held at 43°C. Each of 20 mice received 20,000-25,000 temperature-tolerant amebae installed intranasally. No animals developed disease signs over a 2-week period, and it was concluded that the amebae were nonpathogenic.

It was determined that at least some of the bacteria and amebae in the yellow-brown floc were chlorine resistant. Samples drawn after superchlorination (40-50 mg/1) of both pools in late January showed the floc to be externally bleached; no metazoans or trophic protozoans were present in these samples, but bacterial rods and cocci were evident in yellow central areas within the bleached outer zone. Amebic cysts were also seen in these bleached samples, although numbers were much reduced relative to yellow-brown floc samples. Living amebae (probably *Acanthamoeba*) appeared in the undiluted bleached samples after 48 h.

ACTION TAKEN AND SUBSEQUENT TREATMENT OF POOLS

Both pools were closed on 9 January when preliminary pathogenicity tests were begun at ISU. Filter repairs were completed in the BHP, and water quality in both pools seemed to improve following increased maintenance efforts after the holidays. Lacking firm evidence of health hazards and in consideration of heavy pressure from pool users, a decision was made to reopen the pools on 13 January. Both pools were again closed on 27 January, upon recommendation of the State Board of Health pending results of the CDC pathogenicity studies. While the pools were closed, the following steps were taken to control or eliminate the yellow-brown floc and amebae: 1) both pools were superchlorinated; 40-50 mg/1 residual chlorine was maintained for 24 h; 2) pools were partially drained; 3) all pool surfaces and surge tanks were scrubbed; 4) all recirculation lines were cleaned with a power rooter and water jet; 5) filters in both pools were repaired; 6) in BHP pumping volume was increased and automatic feeding equipment for cationic polymer flocculant, "Cat-Floc-T", installed; 7) after refilling, both pools were "shock chlorinated" (approx. 5-6 mg/1) before being reopened on 10 February. Showering regulations were enforced and an additional full-time maintenance person was employed to regularly vacuum-clean the pools.

Despite these efforts, small amounts of yellow-brown floc reappeared in both pools during April 1981. *Naegleria*-like and vannellid amebae were again common in filter samples. It was suspected that concentration of bromine and chlorine compounds maintained in the pools were not sufficient. Stick bromine compounds (Aquabrome) have been the preferred disinfectants at ISU because of their relative pH independence and reduced eye and skin irritability compared to more commonly used chlorine gas. Bromine compounds were fed into the ISU pools by erosion feeders which may not be as effective as positive displacement solution pump feeders. During summer and fall 1981, the bromine disinfection systems were replaced by automatic liquid chlorination systems which became operational in both pools during late November.

Additional studies of the yellow-brown floc and amebae which persist in the filter systems of both pools are underway at ISU.

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In Memoriam

Dr. Jay Lush, Distinguished Professor, Iowa State University, died May 2, 1982. He was named a Distinguished Fellow of the Iowa Academy of Science in 1975. He was born January 3, 1896 in Page County, Iowa. On December 20, 1923, he married Adaline Lincoln. He received a B.S. (1916) and M.S. (1918) from Kansas State University and a Ph.D. from the University of Wisconsin (1922). He taught high school in Kansas, 1916-19, with time out for U.S. Army Air Service in 1918. He became a research animal husbandman at the Texas Agricultural Experiment Station in 1921, and joined Iowa State University's faculty in 1930.

His research in the genetic and biometric approaches to animal breeding established a scientific basis for the improvement of livestock and earned him an international reputation. He was elected to the National Academy of Sciences in 1967, and in 1969 the National Medal of Science was awarded, the U.S. government's highest award for distinguished service in science, mathematics, and engineering. He received numerous other awards and honorary degrees.