the immediate exciting motives, a deeper analysis will show that after all food has been and is the great problem of the universe. Civilization, as we have it at the present time, is constantly tending to the increase of population at a greater rate than would have taken place under natural conditions. In other words, human evolution has been blocked by artificial methods and, as pointed out, the best stocks, which might be able to solve the grave questions of the future, are not being reproduced in anything like a fair proportion compared to the poorer stocks, which have not shown any capacity for leadership, but which have, in fact, given to the better class some of the gravest problems that have confronted us.

As health officers and administrators, we are obliged to follow the dictates of our religious life, and of civilization which is so largely a product of our religions. We laud the philanthropists who looks towards the alleviation of present suffering rather than to the removal of the causes which may lead to suffering for future generations. We are developing somewhat higher altruistic virtues at the expense of efficiency, and an inexorable law will demand redress in the not distant future. It may be that reforms will come about which will delay the gloomy outcome of our present course, such as sterlization of the unfit, more strict laws regarding the mating of the unfit, birth control, etc. I conceive it to be the duty of health officers and physicians to study all of these questions assiduously; but for the present can only recommend that we continue a program of which we cannot entirely approve from a biological stand-point.

AEROBIC SPORE FORMING LACTOSE FERMENTING ORGANISMS AND THEIR SIGNIFICANCE IN WATER ANALYSIS

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AEROBIC SPORE forming lactose fermenting bacilli are not infrequently encountered in the examination of water for the presence of Bacterium coli. Since these organisms may be responsible for false presumptive tests, it seemed desirable to obtain more data than is now available with respect to their source and their resistance to bactericidal agents.

Meyer[1] in 1918, described an aerobic spore forming lactose fermenting bacillus which he isolated during the course of routine water analysis. Eight strains were obtained from a city water supply and three from a raw tannery waste. The organism was a large Gram negative, nonmotile bacillus with a centrally located spore. Spore formation took place readily. Pink colonies were produced on Endo medium, and gelatin liquefaction began within 48 hours. Acid and gas were formed in dextrose, levulose, raffinose, maltose, sucrose, lactose, inulin, starch, glycerol and mannitol. Dulcite was not attacked. The methyl red reaction was negative and the Voges-Proskauer test was positive. Indol was not formed and nitrates were not reduced. Acid was formed in milk, with coagula-

tion in 48 hours. Meyer regarded this organism as a possible interferer in determining the colon index. Ewing[2] isolated 14 strains from the Baltimore city water supply. The organism was apparently identical with that described by Meyer. The isolations were made in the spring, following a period of heavy rains. Ewing emphasized the necessity of microscopic examination in the confirmation of Bact. coli tests. Ellms[3] reported sporulating lactose fermenting aerobes in the Milwaukee water supply and also isolated strains from fecal material. He described them as Gram positive and methyl red positive. Perry and Monfort[4] isolated 5 strains, apparently similar to those described by Meyer. Hinman and Levine[5] obtained 14 cultures from Iowa waters. Their strains differed from those of Meyer in being motile and Voges-Proskauer negative. Growth was inhibited by bile and by gentian violet. The organism was non-pathogenic when fed to rabbits. Lisk[6] found similar organisms in milk. Roab[7] has reported the organism in water supplies, and Havens and Dehler[8] found apparently similar bacilli in the intestines of fish.

It has been emphasized, particularly by Hinman,[9] that spore forming, lactose fermenting, aerobic bacilli may constitute a real source of error in water analyses.

We first studied the incidence of these organisms in the Chicago water supply (a chlorinated water), and then examined a variety of substances to obtain information on the sources of water contamination.

The following method was employed for isolation and purification. Samples were collected with the usual precautions and inoculated into lactose broth fermentation tubes. The tubes were then heated in a water bath for 15 to 25 minutes at a temperature of 85° to 90° C. After cooling, the tubes were incubated at 37° C. and gas formation observed after 24, 48 and 72 hours. Endo plates were inoculated from each tube showing gas.

From the Endo plates on which red colonies appeared, transfers were made to lactose broth and to agar slant tubes. If gas was again produced in lactose broth, a Gram stain was made from the 24 hour agar growth and motility observed. A spore stain was made from a 48 hour agar growth. If the organism proved to be a lactose fermenting spore former, two lactose broth tubes were inoculated from a 72 hour agar slant growth, the tubes heated at 85° C. for 25 minutes and incubated. The cultures were again purified by the use of Endo plates, and finally transferred to nutrient agar slants.

Table I shows the sources investigated, the number of test samples and the number of strains of lactose fermenting, spore forming aerobic bacilli isolated. Further work on sources is in progress.

TABLE I
INCIDENCE OF ORGANISM

Source	No. Samples	No. Strains	Per cent
Tap	128	2	1.6
Drinking fountains	50	1	2.0
Raw Lake Water	32	1	3.1
Human Urine	6	0	0.0
Human Stools	36	1	2.5
Street Drainage	44	2	4.5
Sidewalk Drainage	10	2	20.0
Snow	4	0	0.0
Mat Dirt	18	2	11.1
Floor Dirt	8	1	12.5
Street Mud	6	2	33.3
Horse Manure	8	3	37.5
Rabbit Manure	6	0	0.0
Sheep Manure	6	1	16.6
Sidewalk Soil	8	0	0.0
Garden Soil	10	3	30.0
Carrot Washings	8	4	50.0
Oat Washings	8	0	0.0
Swimming Pools	36	0	0.0
Raw Sewage	6	0	0.0
Totals	438	25	5.7

This table shows that 438 samples from a variety of sources yielded 25 strains of the bacillus. The tap water had been chlorinated. The water from the drinking fountains was from the same supply, but had been passed through a small sand filter some time after chlorination. The highest incidence of isolation was from animal manures, garden soil and root vegetable washings. The number of recorded isolations is too small to serve as a basis for definite statements regarding the habitat of these organisms. The results are, however, in line with those of other investigators.

The morphologic and biochemic characteristics of the 25 strains were identical. The organisms were rods 3 x 0.6 microns, grouped singly or in pairs. Spores were abundant in 48 hour growths. The spores were centrally located, were oval in shape and measured 1.5 x 2 microns. Motility was observed in all cultures, but capsules could not be demonstrated. The cells stained evenly with gentian violet and were Gram negative. However, the stain was removed with some difficulty. It is not surprising that conflicting reports have been made on this stain.

On plain agar the colonies were irregular in shape, smooth, and showed no distinct internal structure. On Endo medium pink colonies were obtained. The colonies usually had red centers, but lacked the sheen characteristic of colonies of Bacterium coli.

All strains liquefied gelatin in 7 days. In litmus milk, acid, gas and coagulation were produced. The milk tubes closely simulated those obtained with Cl. welchii. Peptonization had started in all but three instances at the end of 7 days. Indol was not produced. The methyl red test was negative and the Voges-Proskauer reaction positive. Fermentation-reactions were observed on 16 carbohydrates. Acid and gas were produced in 48 hours by all strains, from dextrose, lactose, maltose, saccharose, arabinose, xylose, galactose, mannose, levulose, trehalose, raffinose, dextrin, inulin and salicin. Dulcite and adonite were not attacked.

Since spores form readily on lactose agar slants, this medium was used to obtain spores for tests on their resistance toward heat and chemical disinfectants. The spores survived exposure to 100° C. for 45 minutes. Tests were made on resistance of the spores to the action of chlorine, mercuric chloride, phenol and "Lysol." The results are summarized in Table II.

TABLE II
RESISTANCE OF SPORES TO DISINFECTANTS

Disinfectant	Dilution	Time of Exposure (minutes)	Results
Chlorine.....	5/1 million	120	—
	5/10,000	120	—
	5/1 000	10	+
	5/1,000	5	—
Mercuric chloride	1/1,500	20	—
	1/1,000	15	—
	1/500	10	+
	1/500	5	—
Phenol.....	1/50	60	—
	1/20	60	—
"Lysol".....	1/50	60	—
	1/20	60	—

+ Indicates that the spores were killed.
— Indicates that the spores survived.

These tests show that the spores are extremely resistant to the action of disinfectants and indicate that they may be expected to survive chlorination of a water supply.

SUMMARY

The lactose fermenting spore forming aerobic bacilli here described are either identical with or closely related to those isolated by Meyer, Ewing, Hinman and Levine and others.

The sources from which they have been isolated, and the high resistance of their spores to chemical disinfectants, indicate that they may appear at times in drinking water supplies even when the supplies have been chlorinated. Their presence has little sanitary significance. However, in routine water examination these organisms may be responsible for positive colon presumptive tests. In order to eliminate spurious results it may be necessary to proceed with the examination of positive lactose broth tubes according to the standard "completed test." The analyst must use his judgment as to this necessity.

Our studies indicate that these organisms will appear not infrequently in surface water supplies after a rainy period.

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