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THE DESTRUCTION OF MOSQUITOES IN AIRPLANES

A Preliminary Note

By C. L. WILLIAMS, *Senior Surgeon,* and W. C. DREESSEN, *Passed Assistant Surgeon, United States Public Health Service*

For some years the quarantine officials of the world have been concerned over the problem of restricting the spread of yellow fever by means of airplane travel. One important feature of this problem is the prevention of transfer of infected *Aëdes aegypti.*

TRANSFER OF MOSQUITOES

It has been proved by Griffitts [1] that the transfer of mosquitoes by airplane actually occurs. It has been shown that if mosquitoes are placed in airplanes, at least a proportion of them will still be found therein many hours later, although the airplane had flown in the interim some hundreds of miles. Furthermore, a careful search of airplanes has resulted in occasionally finding mosquitoes, including *Aëdes,* that have found their way into the planes at some point along the route.

PROBLEMS OF FUMIGATION

With the finding of mosquitoes (particularly *Aëdes aegypti*) in airplanes demonstrated, it becomes important to devise adequate means of destroying them. At first glance this would appear to be relatively simple, considering the fumigants at present available and the susceptibility of this insect to destruction by fumigation. On closer examination, however, the problem becomes complicated, because of the necessity of applying fumigation at different points. For example, an airplane leaving Rio de Janeiro and stopping at Pernambuco, Port of Spain, San Juan, Port au Prince, Habana, and Miami should be fumigated between each of these stops. Furthermore, fumigation should not be delayed until the arrival of the

[1] Griffitts, T. H. D., and Griffitts, J. J.: Mosquitoes transported by airplanes. Pub. Health Rep., vol. 46, no. 47, Nov. 20, 1931, pp. 2775-2782.

airplane, since during its stay mosquitoes might readily leave before the airplane can be fumigated.

To meet the conditions enumerated, fumigation might be performed at the port of departure and at each port of call immediately before the airplane departs, thereby destroying any mosquitoes taken on at each port; or the airplane might be fumigated while in flight between ports. Both of these conditions present the difficulty that, to some extent at least, the passengers would have to be fumigated along with the mosquitoes.

CHOICE OF FUMIGANT

The apparent necessity for fumigating the passengers along with the mosquitoes eliminates consideration of the most effective fumigants, particularly hydrocyanic acid. Even though the fumigant may be very rapidly cleared from an airplane, the tendency of hydrocyanic acid to become absorbed in upholstery appears to render its use inadvisable for this type of fumigation. The use of HCN during flight, of course, is out of the question.

The thought of carrying out fumigation while the airplane is in flight is an attractive one; obviously, that is the point where fumigation can be applied to the very best advantage. In addition to the passenger fumigation problem, however, the matter of additional weight is presented; the fumigant and the apparatus to apply it cannot be heavy—not more than a very few pounds at the most—without imposing on the carriers a very considerable economic burden.

With these considerations in view, the efforts of the past few months have been directed both toward the utilization of a fumigant relatively harmless to human beings that can be applied while the airplane is at a port of call, and to the development of a fumigant innocuous to human beings that can be carried in small bulk, applied by light machinery, and utilized while the airplane is in flight. The possibilities of the development of the last-named type of fumigant were suggested by the observation that airplanes in which a pyrethrum extract insecticide was sprayed during flight were regularly found free from living mosquitoes on arrival at United States ports.

RANGE OF EXPERIMENTS TO DATE

So far, experimental work has been carried out to a limited extent with carboxide and with concentrated pyrethrum extract.

CARBOXIDE EXPERIMENTS

Carboxide is composed of 1 part ethylene oxide mixed with 9 parts carbon dioxide, packed under high pressure in steel cylinders. It is applied by attaching a pressure hose to the cylinder, leading the hose

into the space to be fumigated, opening the valve wide, and permitting the desired amount of the fumigant to flow out under its own pressure. The amount of gas used is determined by placing the cylinder on scales and noting progressive loss of weight. Carboxide is generally packed in cylinders containing 30 pounds of the fumigant, the cylinder itself weighing something over 40 pounds. It will be noticed at once that the weight of the fumigant and container practically precludes its being carried by the airplane for fumigation during flight.

Ethylene oxide, which is the active ingredient in carboxide, is not dangerous to fumigators handling it during the ordinary course of fumigation in which the fumigators are exposed to the gas for only short periods, and then usually not to high concentrations. It has been shown, however, that when human beings are exposed to this gas for considerable periods or to high concentrations, it is not without effect upon them. Its effect has been tested upon guinea pigs, in which animal it was shown that irritation of the respiratory tract, including the lungs, occurred.

From these considerations it will be seen that the use of carboxide as a fumigant during flight is precluded, but that it might be used for fumigation on the ground followed by rapid ventilation of the airplane immediately before departure.

Experiments to determine the lethal dose of carboxide for *Aëdes aegypti* were carried out during the latter half of 1934 at the New Orleans quarantine station. In these experiments, mosquitoes bred in a colony maintained at the station were exposed to varying concentrations of the gas for varying periods. Briefly, the technique was to capture mosquitoes from the breeding cage by drawing them (by air suction) into a glass tube closed by a wad of cotton, against which the mosquitoes were held. From this tube they were blown into small mosquito netting cages. These cages were placed in a small, carefully sealed room into which the carboxide was blown; the mosquitoes were exposed for the period of the test and then removed to the open air or into a room free from gas. Observations continued for a total period of 1 week following fumigation.

Results are probably best given by citing typical experiments.

Experiment no. 1.—August 29, 1934. 20 *Aëdes aegypti* were exposed for one-half hour to carboxide in a concentration of 10 ounces per 1,000 cubic feet. Result: All mosquitoes alive and active.

Experiment no 2.—August 29, 1934. 30 *Aëdes aegypti* were exposed for 2 hours to 12½ ounces of carboxide per 1,000 cubic feet. Result: Mosquitoes somewhat sluggish when removed from fumigation; next day, all alive.

Experiment no. 3.—September 4, 1934. 37 *Aëdes aegypti* were exposed for one-half hour to 3¼ pounds of carboxide per 1,000 cubic feet. Result: Immediately on removal, 1 mosquito was apparently

dead; the next morning, 3 were dead, others alive; at the end of the week, 30 were dead, 7 still alive.

Experiment no. 4.—September 5, 1934. 23 *Aëdes aegypti* were exposed for 1 hour to 6 pounds of carboxide per 1,000 cubic feet. Result: Immediately after removal, 1 mosquito was apparently dead; the next morning, all but 3 were dead; the day following, all were dead.

Experiment no. 6.—September 7, 1934. 23 *Aëdes aegypti* were exposed for one-half hour to 13¼ pounds of carboxide per 1,000 cubic feet. Result: Immediately on removal, all were alive, the next morning, 4 were still alive, others dead; on the second day, 3 were still alive in the morning, but by afternoon all were dead.

Experiment no. 8.—October 2, 1934. 30 *Aëdes aegypti* were exposed for one-half hour to 12 pounds of carboxide per 1,000 cubic feet. Result: Immediately on removal, 7 were apparently dead; the following day, 16 were dead in the morning, and by evening 23 were noted as dead; the second day, only 6 were still alive; the third day, 3 were still alive, the same number being alive on the fourth day; on the fifth day, 2 were alive, both of which died on the sixth day.

Experiment no. 9.—October 22, 1934. 72 *Aëdes aegypti* were exposed for one-half hour to 15 pounds of carboxide per 1,000 cubic feet. Result: Immediately on removal, 2 were noted as dead; the next day, 68 were dead, leaving 4 alive; the following day, 3 were alive; on the third day, 2 were alive, but were noted as quite weak; on the fourth day, 1 was alive, and this one was found dead on the fifth day.

Experiment no. 12.—October 23, 1934. 64 *Aëdes aegypti* were exposed for one-half hour to 20 pounds of carboxide per 1,000 cubic feet. Result: Immediately on removal from the gas, 7 were noted as dead; 2 hours later, all were dead.

INTERPRETATION OF CARBOXIDE EXPERIMENTS

From the experiments cited, the interpretation is inescapable that mosquitoes (*Aëdes aegypti*) are surprisingly resistant to carboxide. This was quite unexpected, since it is well known that mosquitoes are usually more susceptible to fumigating gases than are most other insect pests. It will be noted that, with one-half hour exposure, the concentration of carboxide necessary to produce death within 24 hours is between 15 and 20 pounds per 1,000 cubic feet of air space.

Exposures longer than one-half hour were ruled out of consideration for practical purposes, since rapid action is essential for utilization in fumigation of airplanes; as a matter of fact, one-half hour is considered far too long a period if it can be at all avoided.

It has been concluded from these experiments that carboxide is not suitable for the destruction of mosquitoes in airplanes.

PYRETHRUM EXTRACT EXPERIMENTS

Pyrethrum extracts have been on the market for years in the form of insecticidal sprays. As a rule, these sprays are made up by diluting a concentrated extract in oil with highly refined kerosene, the dilution generally being such that every 100 cc contains one-tenth gram of pyrethrins.

Since the principal killing agent in these sprays is the pyrethrin, and since the weight of the fumigating material constitutes an essential consideration in the fumigation of airplanes in flight, it appeared to us that there was little use in testing these commercial type insecticide sprays but that it would be much better to work with the concentrated extract from which they were generally manufactured. The one actually selected is an extract, in light oil, of which 1 gallon contains the pyrethrins from 20 pounds of standardized pyrethrum flowers. The flowers are assayed and mixed by the manufacturers so that each 100 cc of the extract contains 2 grams of pyrethrins.

There is available an extract of twice the strength of that just described. It is expected that, in future work, this will be tested.

The method of experiment was to blow the pyrethrum extract into a closed room in the form of a very fine spray, practically a mist. In the first 5 experiments, the amounts were only approximately measured, but thereafter the dosage was accurately determined by weighing the extract container before and after spraying.

Immediately after spraying the extract into the room, two cages of test mosquitoes were placed therein. The room was sealed during the period of exposure. When it was opened, one of the test cages was removed, and it was then closed for an additional exposure period, at the end of which time the second test cage was removed. The mosquitoes thereafter were kept under observation for 24 hours.

All of the results reported herein are based on 24 hours' observation after fumigation. Further observation of the mosquitoes in these tests showed that when 3 grams per 1,000 cubic feet or more of the extract was used, all of the mosquitoes died within 2 or 3 days. Results beyond the 24-hour observation period, however, are not recorded, because it has not as yet been possible to determine definitely whether these fumigated *Aëdes* would attempt to bite before dying. A number of tests of their biting ability after fumigation have been made, all of them negative; but they are too few in number as yet to form the basis of any definite statement.

The results of the 25 experiments with pyrethrum extract that have been performed to date are given in table 1.

TABLE 1.—*Results of 25 experiments with pyrethrum extract*

Experiment no.	Amounts of pyrethrum extract used			Time of exposure	Percent killed in 24 hours	Number of Aëdes	
	Grams per 1,000 cubic feet	Cubic centimeters per 1,000 cubic feet	Ounces (approximately) per 1,000 cubic feet			Total	Female
1	(1)	(1)	5	½ hour	100	40	(1)
2	(1)	(1)	5	½ hour	[2] 100	[2] 50	(1)
				1 hour	100	50	(1)
3	(1)	(1)	2½	15 minutes	100	37	(1)
				½ hour	100	30	(1)
4	(1)	(1)	1½	15 minutes	100	75	31
				½ hour	100	46	22
5	(1)	(1)	½	15 minutes	97	33	14
				½ hour	100	32	16
6	53.0	64.6	2.1	10 minutes	100	42	15
				15 minutes	100	63	41
7	169.5	207.0	6.67	5 minutes	100	44	15
				10 minutes	100	34	14
8	10.0	12.2	⅜	5 minutes	100	31	16
				10 minutes	100	31	18
9	11.5	14.0	.45	5 minutes	100	31	11
				10 minutes	100	20	9
10	15.5	18.9	.6	5 minutes	100	36	15
				10 minutes	100	35	19
11	6.5	7.9	¼	5 minutes	100	39	22
				10 minutes	98	46	28
12	3.3	4.0	⅛	5 minutes	91	47	28
				10 minutes	95	37	25
13	3.4	4.1	⅛	5 minutes	100	39	32
				10 minutes	100	53	24
14	3.9	4.7	1/7	5 minutes	97	67	45
				10 minutes	100	34	18
15	2.5	3.0	1/10	5 minutes	100	52	28
				10 minutes	100	66	23
16	1.97	2.4	1/13	5 minutes	98	47	23
				10 minutes	96	52	31
17	1.11	1.4	1/25	5 minutes	96	51	32
				10 minutes	98	59	39
18	1.04	1.3	1/25	5 minutes	21	63	26
				10 minutes	52	62	38
19	1.25	1.5	1/20	5 minutes	100	50	21
				10 minutes	100	47	13
20	.88	1.1	1/30	5 minutes	66	50	26
				10 minutes	94	65	28
21	2.6	3.2	1/10	5 minutes	93	28	8
				10 minutes	84	88	35
22	1.8	2.2	1/14	5 minutes	99	87	43
				10 minutes	90	84	44
23	1.95	2.4	1/13	5 minutes	95	63	37
				10 minutes	92	73	42
24	2.47	3.0	1/10	5 minutes	98	58	21
				10 minutes	100	83	49
25	2.86	3.5	1/8	5 minutes	100	36	15
				10 minutes	100	34	17

[1] Not accurately measured or counted.
[2] Top figure in each experiment indicates cage given shortest exposure.

INTERPRETATION OF RESULTS

It will be noted in the table that the first 5 experiments are distinctly preliminary. The amount of material used was only approximately determined, and the periods of exposure were relatively long. Having discovered through them, however, that we had an effective fumigating material, the exposures were reduced to 5 and 10 minutes and the amount of material used was progressively made smaller and smaller. Experiments 6, 7, 8, 9, and 10 showed that when 10 or more grams per 1,000 cubic feet were used, with exposures of 5 and 10 minutes, a uniformly 100-percent kill in 24 hours occurred. In experiment 11,

6.5 grams per 1,000 cubic feet produced a 100-percent kill in 5 minutes, though only 98 percent in 10 minutes' exposure. In experiments 12, 13, 14, 15, 21, 24, and 25, in which from 2.5 to 3.9 grams per 1,000 cubic feet were used, the kill was 90 percent or better, with the single exception of 1 portion of experiment 21, where only an 84-percent kill was secured. In 3 of these experiments, numbers 13, 15, and 25, the kill was 100 percent in both sections of the experiment, the amounts used being, respectively, 3.4 grams, 2.5 grams, and 2.86 grams per 1,000 cubic feet.

In experiments 16, 17, 18, 19, 20, 22, and 23, less than 2 grams per 1,000 cubic feet were used; the proportionate kill, however, was better than 90 percent in the majority of these experiments. In one of them, experiment 18, the poor results are believed to have been due to faulty spraying, it being noticed at the time that the spray was much heavier than in other experiments and that a material amount of it was deposited on the floor. In one experiment, number 19, 1.25 grams per 1,000 cubic feet were used, with a kill of 100 percent in both cages. This was the only experiment utilizing less than 2 grams, however, that showed a 100-percent result.

It would appear from these experiments that the minimum dosage of this pyrethrum extract required to kill *Aëdes aegypti* within 24 hours after exposure lies somewhere between 2 and 4 grams per 1,000 cubic feet.

It will be seen throughout these experiments that 5 minutes' exposure produced practically as good results as 10 minutes' exposure.

CONDITIONS OF EXPERIMENTS

The conditions under which mosquitoes were secured for the experiments with pyrethrum extract differed somewhat from those already described. In these, the mosquitoes were bred under control conditions, the larvae being grown in bowls outside of the cages, and the pupae separated and placed in test tubes as soon as they appeared. The test cages were all filled with freshly hatched imagos that appeared in the tubes in which the pupae had been placed. In practically all of the experiments, therefore, mosquitoes between 1 and 3 days old were used. While test cages were in process of being filled, the mosquitoes therein were fed with sugar water.

The test cages used were made of mosquito netting, were cubical in shape, and varied from 6 to 15 inches in each dimension. It is probable that, to a certain extent, the walls of these cages reduced the effectiveness of the insecticide spray by absorbing a portion of it as it passed through them. This point will be checked in later work by fumigating mosquitoes released in the compartment into which the spray is introduced.

Most of these experiments were carried out during the winter months, so that atmospheric conditions were necessarily artificial. The great majority of the experiments were performed in the same building in which the mosquito colony was maintained, which building was kept at a temperature of between 75° and 85° F. Humidity sufficient to prevent material loss in the colony was maintained by hanging wet blankets in the room, placing pans of water on the radiators, and keeping a pot of water over an electric hotplate. The relative humidity, however, was not determined.

It is expected that the results reported will be checked during the summer months under outside atmospheric conditions, which, in the climate of New Orleans, are favorable to the propagation and maintenance of *Aëdes aegypti*.

The mosquito colony was subject to frequent check by examination of individuals and by examination of the mosquitoes used in these experiments. It was maintained throughout as a pure colony of *Aëdes aegypti*.

MAINTENANCE OF THE MOSQUITO COLONY

Briefly, the colony of *Aëdes aegypti* was maintained by inoculating and maintaining breeding in 5 wire-mesh cages, cubical in shape, and approximately 3 to 4 feet on a side. In some of the cages the mosquitoes were permitted to lay eggs on damp sponges, which were taken out, allowed to stand for a day or so, and then placed in water to permit the eggs to hatch. In others, small cypress water-troughs were placed, these proving attractive locations for the deposit of eggs; the eggs appeared just above the water-line; and, as the water evaporated, more and more space for them became available. At the end of 10 days, the troughs were removed and water was added, filling them to the brim, when the eggs in the troughs promptly hatched.

All larvae were transferred to china bowls and fed on small amounts of brewer's yeast. When the pupae appeared, they were removed with a large-mouthed medicine dropper to test tubes containing water. When the pupae hatched, the imagos were in part returned to the breeding cages and in part used for experiments.

Mosquitoes in the cages fed on clipped rabbits every 2 days; in the interim they were allowed to feed on sugar water absorbed in cotton sponges.

It was found necessary to place the supporting legs of the cages in pans of water covered with kerosene oil to prevent ants from carrying away the mosquito eggs, while daily careful searches of the cages were required to eliminate small house spiders, which were the principal enemies of the adult mosquitoes.

TENTATIVE CONCLUSIONS

1. Carboxide is not a suitable fumigant to kill mosquitoes in airplanes, either in flight or on the ground, because the containers are too heavy and the amount of material necessary to kill an effective percentage of *Aëdes aegypti* is too large.

2. A concentrated oil extract of pyrethrum flowers containing 2 grams of pyrethrins per 100 cc is highly effective against *Aëdes aegypti* when brought in contact with them in the form of a very fine spray, the lethal concentration apparently being somewhere between 2 and 4 grams per 1,000 cubic feet.

3. Mosquitoes fumigated with either carboxide or pyrethrum extract do not die at once. It must remain for future experimentation to determine whether they are rendered incapable of biting before dying.

4. The small amount of concentrated pyrethrum extract required to kill mosquitoes should render this material suitable for the destruction of these mosquitoes on airplanes in flight.

5. It is the general belief that neither the pyrethrins nor the oil in which they are dissolved is harmful to human beings.

STUDIES OF SEWAGE PURIFICATION

II. A ZOOGLEA-FORMING BACTERIUM ISOLATED FROM ACTIVATED SLUDGE

By C. T. Butterfield, *Principal Bacteriologist, United States Public Health Service, Stream Pollution Investigations, Cincinnati, Ohio*

BRIEF REVIEW OF THE LITERATURE

Research studies on the activated sludge process conducted by the Stream Pollution Investigations Laboratory of the United States Public Health Service during the past 2 years, under the direction of Sanitary Engineer J. K. Hoskins, have indicated that zoogleal material is regularly present in large amounts in activated sludge flocs. Each time that an activated sludge has been developed during this study, regardless of whether it was in a small laboratory set-up or in a tank of plant-size proportions, the floc developed has contained a very considerable amount of zoogleа. When the process was working most efficiently, zoogleal masses predominated in the sludge. These findings, which are in general accord with the observations made by previous workers, point to the very probable importance of this type of organism and suggest an intensive study of the zoogleal bacteria found in activated sludge to determine their characteristics and the efficiency of any sludge produced by them under pure culture conditions.

The earliest report found, that referred to zoogleal bacteria, was the book of Flügge (1886) on micro-organisms. Kruse, who wrote a chapter in this text, mentions zoogleal bacteria and states that Itzigsohn, in 1867, was the first to observe zoogleal formations. Itzigsohn designated the specimen studied by him as *Zooglea ramigera*. Kruse states further that Zopf considers this *Zooglea ramigera* to be one phase in the life cycle of Cladothrix.

Winogradsky (1892) noted that the nitrite-forming bacteria existed both in the motile and in the zoogleal stage. He believed that the zoogleal form probably represented a resting stage. This observation in regard to a resting stage may be true in the case of nitrifying bacteria, but our results would indicate that, under the conditions of our experiments, or in activated sludge, the zoogleal stage, while immotile, is a very active phase in terms of the utilization of food material. This observation of Winogradsky on the development of a zoogleal stage by the nitrifying bacteria would seem to suggest definitely that this type of zoogleal mass may adsorb unoxidized ammonia compounds just as the zooglea reported later in this paper removed other oxidizable material from solution. Indeed, it is not unreasonable to assume that a number of bacteria of this type which produce sludges of various adsorptive properties may be located within this group, and efforts to isolate and study such organisms are indicated.

Flügge (1896), discussing the development of zooglea, believes that the zoogleal matrix is a massed exhibition of capsular substance. He states that a given zoogleal mass is generally entirely composed of the same type of cell, suggesting a pure culture. He finds that in polluted water the growth of zoogleal masses is rapid; that both spherical zooglea and a branched tree-like form, called *Zooglea ramigera*, are very common. His illustration (fig. 13) of this tree-like form is quite similar to the specimen shown in our figure 2. Unfortunately, no further characteristics of the organism are given. He credits Kruse with having originally assigned the name of *Zooglea ramigera* to this type of growth.

Stützer and Hartleb (1897), in their series of papers on the saltpeter fungi, give a limited description of *Zooglea ramigera*. They describe it as forming spores, oxidizing nitrogen compounds, and as manifesting exceedingly variable morphological characters, including a coccoid, rod, and fungus-like stage, as well as the zoogleal formation. In addition, they found that, in the latter stages of growth, this organism would reduce both the nitrites and the nitrates which had been formed. These marked variations, together with their definite disagreement with the researches of Winogradsky on the nitrifying bacteria, whose findings have since been fully substantiated, leads one to question the purity of the cultures employed by them.

Some uncertainty appears to exist as to whether the gelatinous matrix of the zoogleal mass has been synthesized by the bacteria or whether the bacteria are simply embedded in a chemically preformed matrix. Zooglea-forming bacteria have been investigated by various workers in studies of gum formation. Thus, in his work on *Rhizobium radicicolum*, Buchanan (1909) found that this organism produced a gum and existed in part as a zoogleal mass. In discussing this he states: "It seems but fair to conclude from this array of evidence that there is a great possibility that bacterial slimes and gums of whatever kind are produced as a transformation or solution of the bacterial capsule." Again, Buchanan (1922) defines zooglea as bacteria growing in masses of gelatinous material secreted by the bacterial cells and shows two figures demonstrating the individual bacteria.

Waksman (1927), in discussing this group of organisms, states: "The gum is formed with various sources of energy in the medium, such as cane sugar, glycerol, or legume extract, and should be considered as a synthesized product."

Löhnis (1921), writing of zoogleal bacteria in general, indicates that large encysted agglomerations of bacterial cells are not at all uncommon and implies further that the term zooglea has been so generally and so loosely used for all slimy agglomerations of bacteria that it has lost its original specific meaning.

Theories regarding the role of the bacteria in sewage purification processes have varied from the opinion that they are essential to the process, no purification being accomplished without their activity, to the view that their presence is entirely unnecessary, the purification observed being an illustration of chemical catalysis.

Johnson (1914) was apparently the first observer to emphasize the importance of the bacteria in the activated sludge process. Writing of sewage filters, he states: "The filter material rapidly becomes coated with a slimy or gelatinous growth of *Zooglea ramigera*, which may be regarded as a large number of bacteria embedded in a gelatinous matrix. This zooglea is perhaps the most characteristic and important organism of this zone." Again, referring on this occasion to activated sludge, he suggests that microscopically activated sludge repeatedly contained Opercularia and zooglea. He believes that the zooglea assisted by the protozoa are responsible for the rapid purification accomplished.

Buswell and Lang (1923) presented results on the microbiology of activated sludge and advanced a theory concerning the process. Since then, Buswell (1928, 1931) has made extensive observations on the gross biology of activated sludge and he is convinced that

the zoogleal bacteria, *Zooglea ramigera*, and the protozoa are of primary importance in activated sludge. The pure culture studies necessary to establish this relationship and to determine the cultural characteristics were not made. In 1928, after presenting the results of his own studies and reviewing the literature on the subject, Buswell, in summarizing, suggests the following statement as the theory of the activated sludge process: "Activated sludge flocs are composed of a synthetic gelatinous matrix similar to that of Nostoc, or Merismopedia, in which filamentous and unicellular bacteria are embedded and on which various protozoa and some metazoa crawl and feed. The purification is accomplished by ingestion and assimilation by organisms of the organic matter in the sewage and its resynthesis into living material of the flocs. This process changes organic matter from colloidal and dissolved states of dispersion to a state in which it will settle out."

As food material, before it can be assimilated and used for energy or be synthesized into protoplasm, must pass through the bacterial cell wall, the extent of bacterial surface available is an all important factor in studying the purification accomplished by bacteria. Buswell calculates that, considered on the basis of the zoogleal masses alone, each cubic foot of the aeration chamber contains at least 250 square feet of such surface. If the surface of the free-swimming bacteria and of the protozoa were included, he believes that approximately 500 square feet of surface would be provided per cubic foot of aeration chamber.

Taylor (1930) concurs with Buswell's theory and states: "A close examination under high powers of the microscope reveals that the bulk of the sludge is composed of jelly-like masses, in which bacteria are present in large numbers. This is a typical zooglea formation, caused by the fusion of the gelatinous sheaths surrounding the bacterial cells. This zooglea is apparently the only growth in activated sludge which is a constant factor. The usual form of the zooglea is that of an irregular, lobed mass, but at times a branching, or filamentous form predominates."

Many other writers have commented on zoogleal growths in sewage purification processes without offering any specific data regarding the characteristics of these bacteria or the sludges produced by them in pure culture. Bergey (1934), in his Manual of Determinative Bacteriology, does not list *Zooglea ramigera* or any bacterium with definite zoogleal characteristics. This omission is due probably to the paucity of definite evidence for the differentiation of this type of organism. Intensive effort has been directed, therefore, to the isolation and study of zoogleal bacteria from activated sludge.

METHODS OF PRESENT STUDY

Initial efforts to isolate the zoeglea-forming bacteria were made by picking colonies from routine standard agar plates made from activated sludge. These colonies were transferred to nutrient broth, incubated with and without aeration and studied to determine the presence of zooglea formation. No such growths were obtained although hundreds of colonies were picked and examined.

Solid media containing gelatin in the place of agar were also tried without success. A special sludge agar was then prepared in which the distilled water ordinarily employed in the preparation of media was replaced with fresh sewage-activated sludge mixtures. It was thought that this would supply the plating media with all of the ingredients found in the sludge where the zoogleal bacteria were known to grow well. However, although large numbers of colonies were picked and studied, no zooglea-forming bacteria were obtained.

Effort was then directed to the purification of clumps of zooglea prior to planting to render the sample suitable for examination in liquid media. Selected typical zoogleal formations, such as those in the unstained preparation from normal activated sludge, illustrated in the photomicrograph in figure 1, were picked out with sterile capillary-tipped pipettes and transferred in series through dilution waters in an attempt to wash them free from extraneous bacteria and foreign matter. This method had been successfully employed previously for the isolation of plankton. A zoogleal mass illustrating both the fingered and the solid type of formation produced by this organism, washed fairly free from extraneous material, is shown in the photomicrograph in figure 2.

In carrying out this cleansing procedure an unexpected phenomenon was encountered. During the course of the washing, the embedded bacterial cells would free themselves from the gelatinous matrix and move away with incredible speed, dispersing throughout the dilution water, long before a satisfactory washing had been accomplished. Such a dispersal of embedded cells is shown in figure 3. In this photomicrograph the faint outline of the zoogleal matrix may be seen with cells scattered without as well as within its limits. In figure 4, such a gelatinous zoogleal matrix, which has been fixed with a mordant and stained, may be clearly observed. Observations made subsequently appeared to suggest that the embedded bacteria feed on nutrient material adsorbed by the gelatinous matrix and then, when it is cleared of such material by repeated washing, they leave the matrix possibly in an effort to find sufficient food.

It was found that this dispersing action could be prevented by the addition of a considerable amount of dissolved organic material to the wash water. Ten cc of sterile nutrient broth added to 90 cc of dilution water proved to be satisfactory for this purpose. Using such a water a zoogleal mass could be washed through 10 to 12 changes of water until it was entirely clear of extraneous material and apparently free from contaminating bacteria. The clump was transferred at this time to normal dilution water and changes in such water continued until dispersion of the cells occurred. This suspension was then placed in a measured amount of sterile dilution water and planted on standard agar plates and in serial dilutions in standard lactose broth.

No colonies containing zooglea-forming bacteria were obtained from the agar plates. In fact, only a few colonies of any kind were observed and they were confined to the 1-cc and the 0.1-cc plates made from the original suspensions. Growths of zooglea-forming bacteria were obtained from all broth tubes up to and including the 1-to-100,000 dilution from tubes incubated at both 20° and 37° C. The growths obtained appeared to be in pure culture in all tubes above the 1-to-100 dilution. This process was repeated several times and plantings were made in each case from the highest dilution showing growth, with intermediate washing in dilution water, to insure the purity of the culture before intensive studies were undertaken. The photomicrograph shown in figure 5 portrays a typical growth taken from one of these broth cultures. The granular nature of this floc is clearly illustrated in the figure. In figure 6 a portion of the floc found in the left center section of figure 5 is shown under higher magnification. Here the bacterial nature of the floc can also be observed. This culture was designated as Z–1.

A second isolation, using the same procedure, was made from a normal activated sludge produced in another series of experiments. The organism isolated at this time was apparently identical in all respects with the bacterium obtained from the first isolation. This culture was designated as Z–3.

After experimental results, reported later in this paper, had shown that this zoogleal bacterium was probably of especial significance in sewage purification and in the activated sludge process in particular, a detailed study was made of the conditions favoring the growth of this organism and of its characteristics.

CHARACTERISTICS OF THE ZOOGLEA-FORMING BACTERIUM

Morphological.—This organism is rod-shaped, average length 3 microns, varying from 2 to 4, average diameter 1.5 microns, varying from 1 to 2, with definitely rounded ends. In liquid media it shows a marked tendency to grow in a floc or zoogleal mass manifested as a loosely bound floc, as a dense spherical mass, as an evenly lobed mass,

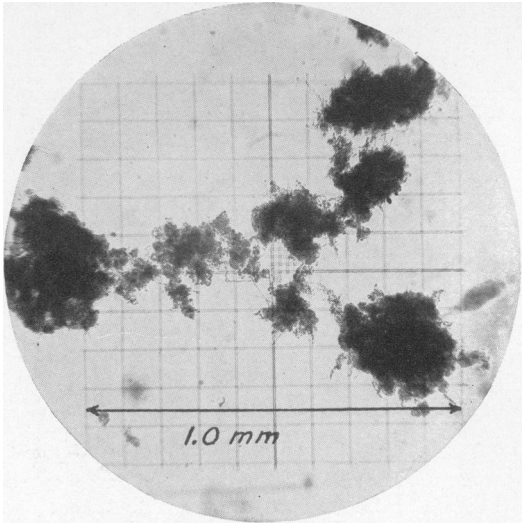

FIGURE 1.—Activated sludge, showing flocs with typical zoogleal formations (unstained).

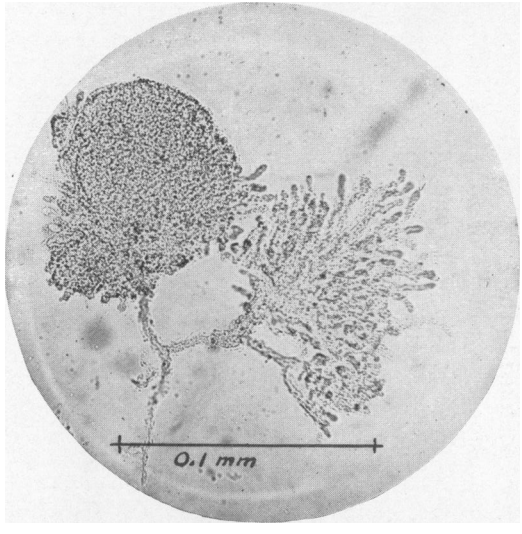

FIGURE 2.—Zoogleal mass from normal activated sludge floc, washed and teased free from nonzoogleal material (unstained).

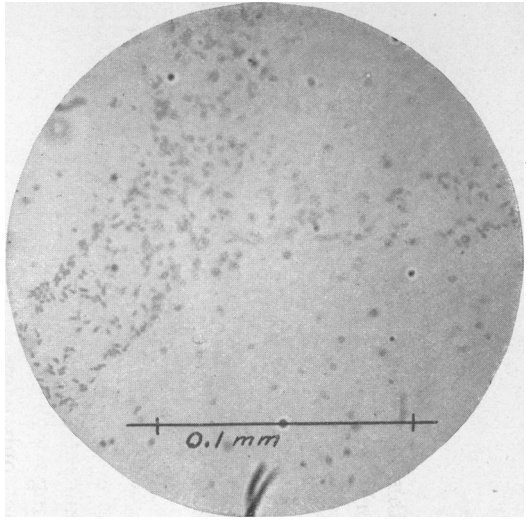

FIGURE 3.—Partially dispersed zoogleal mass, showing bacteria both without and within the matrix (unstained).

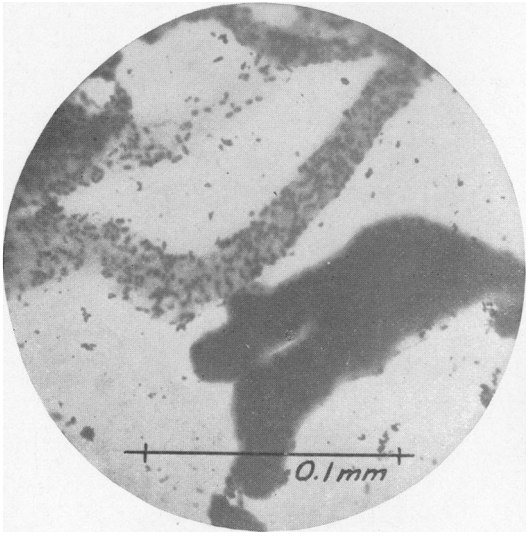

FIGURE 4.—Zoogleal masses stained after fixation of the matrix with mordant. In the central mass, from which many of the bacteria have dispersed, the matrix can be clearly seen.

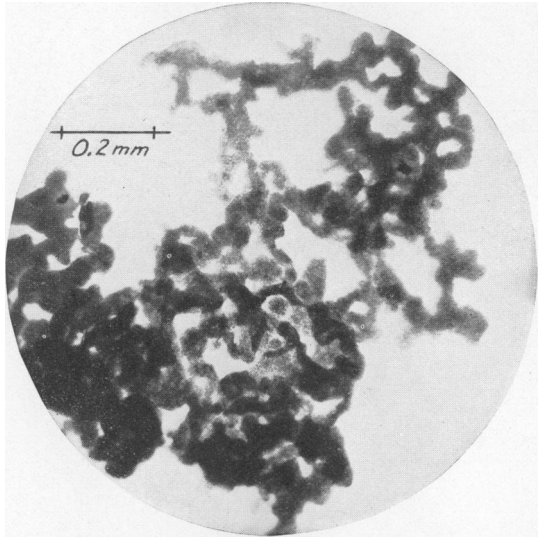

FIGURE 5.—Zoogleal mass from pure culture growth in broth (stained). Low power.

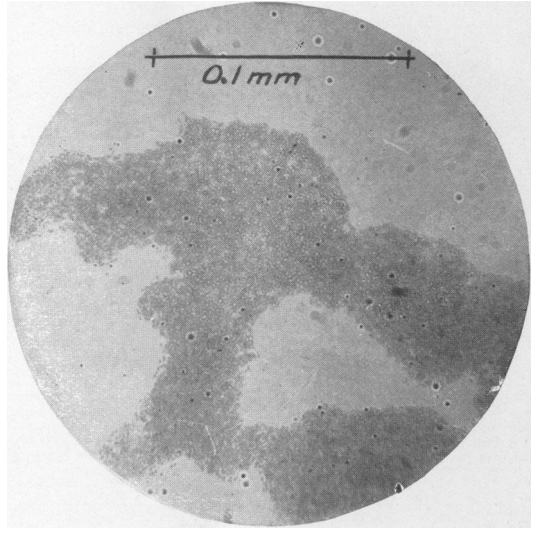

FIGURE 6.—Zoogleal mass from pure culture in broth. High power. Tip of floc shown in upper left of figure 5.

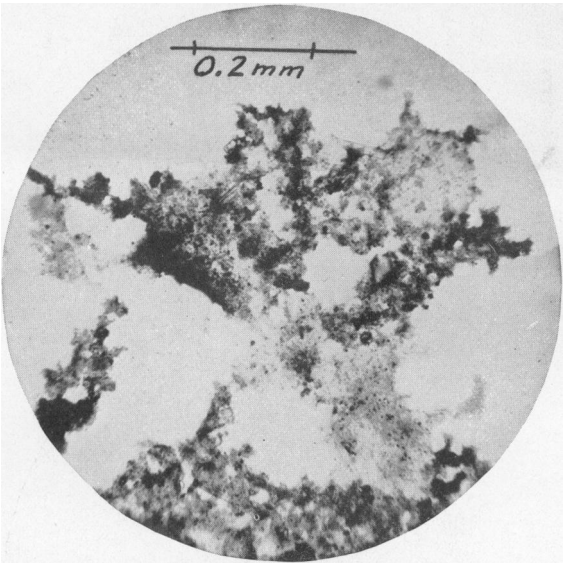

FIGURE 7.—Activated sludge developed by zoogleal bacteria in pure culture in sterilized sewage.

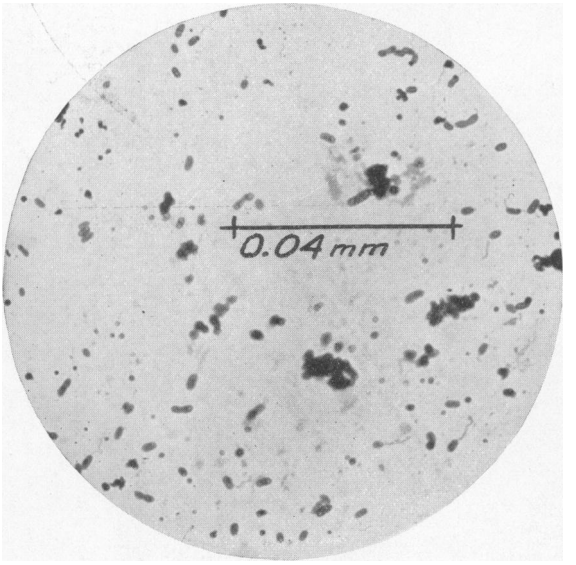

FIGURE 8.—Zoogleal bacteria. Flagella stain of thick smear.

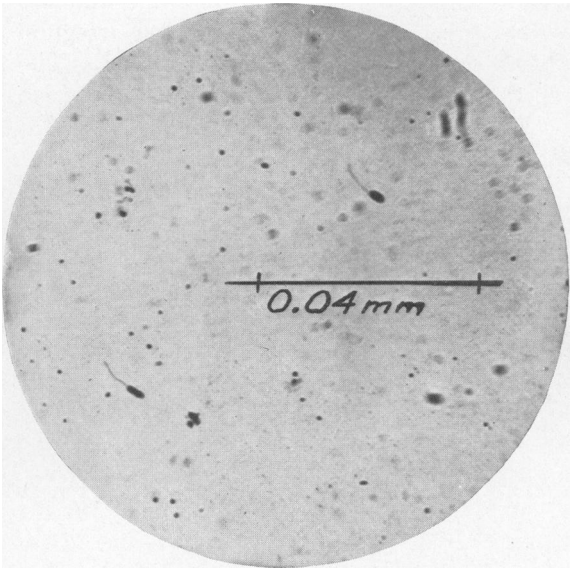

FIGURE 9.—Zoogleal bacteria. Flagella stain of thin smear.

or as a fingered treelike floc. When found outside the mass it usually occurs singly, occasionally in pairs, or in fours joined end to end. Capsules were always observed when stained by the method of Anthony (1931). The capsular wall was from 1 to 1½ times as thick as the bacterium. Spores are not produced, as none have been observed microscopically and no growth was ever obtained after application of the heat test. When not embedded in a zoogleal mass, individual organisms are very actively motile. Chains of 2 to 4 are motile but not as active as individual cells. The bacterium possesses a single polar flagellum about 5 to 6 microns long. This character, as demonstrated by the staining method of Gray (1926), is shown in figures 8 and 9. Although the usual variation in the size of cells was noted, irregular or involution forms were not found in the cultures examined. The organism was not observed to retain Gram's stain at any stage of culture.

Cultural.—No growth of this organism has been obtained at any time on standard agar or gelatin. Scanty growth was obtained on a special sludge agar and a moderate growth on nutrient agar containing 10 percent ascites fluid. It grows well at both 20° and 37° C. in nutrient broths containing peptone, producing a flocculent growth with an abundant sediment which is flocculent to granular. As a rule, the broth without the flocs remains clear and is free from odor. Broth with an initial pH of 7.0 to 7.2 has invariably become more alkaline as growth progresses, until in 5 days at 37° and in 10 days at 20° a pH of 8.2 to 8.4 is reached. It grows luxuriantly in sterilized sewage under aeration, producing a flocculent sludge which settles rapidly when the agitation is stopped. The alkalinity of such sewage is increased by this growth until a pH of 8.6 to 8.8 is reached.

Physiological.—A slow growth of this organism has been observed at temperatures as low as 4° C. Most vigorous multiplication occurs between 20° and 37° C., with the optimum temperature approximately at 28° to 30°. Growth takes place over a pH range of 5.6 to 8.5. However, the rate of growth at the lower figure is very slow until the pH has been raised by the products of growth. The optimum pH appears to be at about 7.0 to 7.4. No evidence of pigment production has been noted. Indole is not produced in either peptone or tryptophane broth. These tests for indole were made at 2 and 10 days, using the technique of both Böhme (1905) and of Goré (1921). Hydrogen sulphide was not produced. The organism is a strict aerobe, failing to grow, during a 10-day incubation period, in vacuo or when the oxygen of the air has been replaced by nitrogen. The organisms subjected to such anaerobic conditions for 7 days were not killed, however; for when half of the cultures were removed and placed under aerobic conditions, growth occurred within 24 hours. Tests were made on the ability of this organism to ferment glucose, lēvulose, lactose, sucrose, maltose, mannite, innulin, and xylose when present in

standard broth and in peptone-free synthetic media. No growth was observed in the synthetic media. Good growth occurred in the presence of each of these sugars in nutrient broth. No gas was produced in any instance; and, as evidenced by changes in the hydrogen-ion concentration, no acid was produced from any of these sugars. However, acid may have been produced and neutralized by the byproducts of growth; for, as has been noted previously, this organism produces alkali in nutrient broth until a pH of about 8.3 is reached, while in these sugar broths a pH of 7.7 was the maximum reached in all cases after 10 days.

The survey made of the literature has not revealed a definite description of an organism with the characteristics of this zoogleal bacterium. However, regardless of the fact that this organism may manifest a growth formation of dispersed single cells, of loosely bound flocs, or of dense spherical and lobed masses, depending on various factors, the peculiar branched treelike form of zoogleal growth assigned to *Zooglea ramigera* by Flügge, together with the illustration given by him, is most unusual and is, under certain conditions, a characteristic of the organism described here. While such limited information as this is too meager to warrant any attempt at the classification of an organism with ordinary characteristics, it is believed that, in the case of this bacterium with this peculiarly shaped colonial form in liquid media, this one characteristic is almost sufficient for identification. For this reason this zoogleal organism, which has now been somewhat more fully described, is tentatively assumed to be a variety of the *Zooglea ramigera*, named by earlier workers, until further studies may confirm or disprove this belief.

EXPERIMENTAL RESULTS

With synthetic media.—Experiments were instituted now with this zoogleal organism Z–1 to determine (*a*) whether it would produce a sludge under conditions of activation and (*b*) the properties of such sludges if any were produced. These tests were carried out under aseptic conditions and with reproducible synthetic media to eliminate as many variables as possible. Dilute standard lactose broth prepared by diluting 6 cc of broth to 100 cc with dilution water was used as synthetic media. Such media have a 5-day biochemical oxygen demand of about 300 parts per million, simulating in this respect a fairly strong domestic sewage. Sterilization by autoclave was carried out in containers, put up with the necessary air-filters and appliances to provide for continuous aeration under pure culture conditions.

Preliminary trials made with this set-up disclosed that a good sludge floc was formed under conditions of very moderate aeration, but when air was applied at a normal rate the floc tended to break up and become dispersed. This, in connection with the observed make-

up of normal activated sludge flocs, appeared to indicate the need for some inert foreign substances to act as a binder or framework for the floc. A number of substances were considered for this purpose. Very short cotton fibers and asbestos fibers were selected for trial because of their inert character. Initial tests with these materials showed that the zoogleal bacteria would develop rather tenacious flocs adherent to cotton fibers, but they would not adhere to asbestos fibers under any of the conditions tried.

The aeration apparatus was then set up with the synthetic media prepared with the addition of about 0.2 gram of short cotton fibers per liter of media. It was inoculated with the zoogleal organism and aerated continuously. After 48 hours a definite floc formation was observed. Thereafter, during the course of these tests this floc was allowed to settle for 30 minutes twice daily. At each settling two-thirds of the contents were siphoned off as supernatant and were replaced with a like amount of the original media. This process was continued until a sludge of 2,000 parts per million or over, measured in terms of suspended matter, had been developed. This sludge settled rapidly with a sludge index of 15 to 20 (15 to 20 percent of the sewage-sludge mixture) at the end of 30 minutes' settling.

Tests were then made on the capacity of this sludge to remove oxidizable material from solution. This observation was made by taking first a sample of the supernatant for a biochemical oxygen demand determination, immediately after fresh media had been added and thoroughly mixed. The mixture was then aerated for a 3-hour interval and a sample of supernatant was removed for a second biochemical oxygen demand determination. The difference between these two oxygen demand results should represent any changes which had occurred in the supernatant during the 3-hour aeration period. That is, if any oxidizable material had been removed from the supernatant by aeration of the media with the sludge, the oxygen demand of the second sample should be correspondingly reduced. To avoid the errors of unbalanced biological activity, which are frequently encountered when sterile or pure culture samples are put up for oxygen demand determinations, these samples were heavily seeded with a grossly mixed culture of bacteria and plankton. Each pair of samples was seeded with the same mixed culture and to the same extent.

Five such tests were completed with this experimental set-up. The amount of oxidizable material removed during the 3-hour aeration interval, as indicated by the biochemical oxygen demand determination, was, for the five tests, as follows: 75, 76, 66, 68, and 73 percent, respectively.

Control experiments using the same synthetic media containing cotton fibers, with nonzoogleal bacteria present and without bacteria,

were carried on at the same time and under the same conditions. No sludges were developed either with or without bacterial growth. The cotton fibers under these conditions did not settle materially during a 30-minute period. In the series with bacteria, *Bact. aerogenes* was introduced and other bacteria from the air gained entrance. A very marked bacterial growth developed. However, oxygen demand determinations on samples collected before and after a 3-hour aeration period, after fresh media had been added as in the test series, did not in any instance show that any appreciable amount of oxidizable material had been removed. In fact, the removal indicated did not exceed 5 percent in any test.

The results show definitely that, under the conditions of these tests, the activated sludge formed by this zoogleal organism is a potent factor in the removal of oxidizable material from solution, removing an average of 72 percent during a 3-hour period. This is very greatly in excess of the amount that could be oxidized during such an interval by the usual biochemical process.

With sterilized sewage.—The tests described above, conducted with synthetic preparations, are subject to the criticism that the media did not correspond to sewage and that the zoogleal sludge formed may have been peculiarly adapted to adsorb ingredients of the synthetic media. To meet such criticism and more closely to simulate the conditions in a sewage plant, in the following-described tests a change was made from synthetic media to natural sewage.

To maintain pure culture conditions which are essential if the results obtained are to be ascribed solely to the activity of the zoogleal bacteria, it was necessary to sterilize the sewage prior to use. Sterilization by chemical means was not suitable, as any substance which would adequately sterilize the sewage could not be removed completely. Sterilization by filtration was not satisfactory; for not only would the sewage be materially altered by such filtration, but also it would be impossible to state positively that all biological elements had been eliminated. Sterilization by heat was adopted, therefore, as the most satisfactory procedure. Catch samples of domestic sewage were collected from day to day and autoclaved for the media in the tests made. The strength of this sewage varied greatly, but a large enough sample was collected each time so that all units of a series, including the controls, could be started or dosed with identical media. This autoclaving of the sewage consistently shifted the hydrogen-ion concentration from about pH 7.2 to about pH 9.0. Before use, the sterilized sewage was always adjusted to pH 7.0 with sterile 1 : 10 phosphoric acid.

Using such sterilized sewage as media in 8-liter amounts and sterile apparatus designed to permit aeration under aseptic conditions, 4

sludges were developed. A good floc simulating activated sludge began to appear after 48 hours' aeration. Thereafter, during the course of the tests, 5 liters of supernatant, after 30 minutes' settling, were removed daily (with the exception of Sundays) and a like amount of sterile sewage was added. After about 2 weeks a sludge of 2,000 parts per million which settled rapidly with indices between 15 and 20, had developed. Biochemical oxygen demand tests to determine the amount of oxidizable material removed from the supernatant during a 3-hour aeration interval after additional sterile sewage had been added were made in each case. The results showed that 68, 76, 66, and 64 percent (average 68 percent), respectively, of the oxidizable material in the supernatant had been removed.

In one container of this series, after the tests had been completed the mixture was divided into two parts, maintaining pure culture conditions, and an inoculation of a bacteria-free protozoa culture, Colpidium, was made into one of them. Both parts, thereafter, were treated in the same manner and the supernatant was replaced with fresh, sterile sewage daily until the Colpidium had reached their maximum number. Oxygen demand tests were then made on the supernatant before and after a 3-hour aeration period. The tests showed that the percentage of oxidizable material removed by the zooglea plus Colpidium mixture was only slightly greater than that obtained with the zooglea-only sludge. However, the supernatant from the aeration vessel containing the zooglea plus Colpidium was much clearer. Microscopical examinations showed that the majority of the free-swimming bacterial cells and the zooglea of microscopic proportions had been cleared from this effluent.

These observations with sterilized sewage as test media were repeated. In this set-up zoogleal cultures Z–1 and Z–3 were both used. A separate container was employed for each culture. A sludge of good appearance had developed in each after 3 days' aeration. Thereafter, 5,000 cc (out of 8,000 cc total) of supernatant were withdrawn daily and replaced with sterile sewage. After 10 days sufficient sludge had developed to begin observations. A photomicrograph of this sludge is shown in figure 7. In this series, suspended solids and oxygen demand observations were made on all samples collected both before and after the 3-hour aeration period. Occasional observations were also made on the nitrite and nitrate content of the aeration mixtures. No material change in the amounts of either of these substances present was observed. This would suggest that this organism did not oxidize ammonia to nitrites or nitrites to nitrates. The results of this series of tests are presented in table 1.

TABLE 1.—*Data concerning activated sludges developed by zooglea cultures Z–1 and Z–3*

Culture	Suspended solids, parts per million, sample collected—		Changes during 3-hour aeration period	
	Before aeration	After aeration	In suspended solids	In percent oxidizable material removed from supernatant
Z–1	1,600	1,800	+200	69
Z–1	[1] 4,100	3,776	−324	41
Z–1	6,512	7,032	+520	71
Z–3	1,810	2,333	+523	57
Z–3	2,248	2,520	+272	68
Z–3	4,528	4,712	+184	56

[1] A large dense particle of debris was observed in this crucible after the weighing had been made.

After these tests had been completed, a mixture of Z–1 and Z–3 sludge was divided into two parts. One was inoculated with Colpidium and both were continued in regular operation until the Colpidium had reached a number of 53,000 per cc. A test was then made to determine the amount of oxidizable material removed during a 3-hour aeration interval. In this instance the zooglea plus Colpidium sludge was more effective, removing 84 percent while the zooglea alone removed 65. It was again observed that the supernatant from the Colpidium-containing mixture was much clearer.

The results given in table 1 indicate again that a large amount of the oxidizable material in the added sewage is removed by the zoogleal sludge during a 3-hour aeration period. In addition it is noted that, in 5 out of 6 of the suspended solids tests made in this series, the weight of the sludge in terms of suspended matter materially increased during the 3-hour aeration interval, indicating an adsorption of dissolved and perhaps of colloidal material.

In connection with these experiments with sterilized sewage as media, control tests were run with sterile sewage under aseptic conditions and with sterilized sewage inoculated with *Bact. aerogenes* and treated in the same manner as those inoculated with zooglea. Oxygen demand observations before and after a 3-hour aeration period were made on these control experiments only after the sewage had been under aeration for a number of days and the *Bact. aerogenes* had developed fully. The results of these control tests showed that no oxidizable material was removed during a 3-hour period either in the sterile container or in the vessel containing *Bact. aerogenes*. In one instance a 7-percent increase in oxidizable material was observed, and in another a 5-percent decrease. These amounts are well within the limits of error for an individual determination of this type.

SUMMARY

Zoogleal masses have been observed in every good activated sludge examined. This conforms with earlier reports in the literature.

A zooglea-forming bacterium has been isolated in pure culture from activated sludge.

This bacterium in pure culture, both in synthetic media and in sterilized sewage has produced a floc which simulated activated sludge.

This pure culture floc has been shown to remove, during a 3-hour aeration period, from 41 to 84 percent of the oxidizable material present in polluted water.

The morphological, cultural, and physiological characteristics of this bacterium are given in detail.

This organism is tentatively identified as a variety of *Zooglea ramigera* as described by earlier workers.

It is suggested that an adequate knowledge of this and related organisms may be of considerable significance in sewage purification processes depending on biological activity.

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DEATHS DURING WEEK ENDED APRIL 27, 1935

[From the Weekly Health Index, issued by the Bureau of the Census, Department of Commerce]

	Week ended Apr. 27, 1935	Corresponding week, 1934
Data from 86 large cities of the United States:		
Total deaths	9,017	8,609
Deaths per 1,000 population, annual basis	12.6	12.0
Deaths under 1 year of age	607	645
Deaths under 1 year of age per 1,000 estimated live births	56	60
Deaths per 1,000 population, annual basis, first 17 weeks of year	12.6	12.6
Data from industrial insurance companies:		
Policies in force	67,826,175	67,729,876
Number of death claims	14,265	13,953
Death claims per 1,000 policies in force, annual rate	11.0	10.7
Death claims per 1,000 policies, first 17 weeks of year, annual rate	10.7	11.1

PREVALENCE OF DISEASE

No health department, State or local, can effectively prevent or control disease without knowledge of when, where, and under what conditions cases are occurring

UNITED STATES

CURRENT WEEKLY STATE REPORTS

These reports are preliminary, and the figures are subject to change when later returns are received by the State health officers

Reports for Weeks Ended May 4, 1935 and May 5, 1934

Cases of certain communicable diseases reported by telegraph by State health officers for weeks ended May 4, 1935, and May 5, 1934

Division and State	Diphtheria		Influenza		Measles		Meningococcus meningitis	
	Week ended May 4, 1935	Week ended May 5, 1934	Week ended May 4, 1935	Week ended May 5, 1934	Week ended May 4, 1935	Week ended May 5, 1934	Week ended May 4, 1935	Week ended May 5, 1934
New England States:								
Maine	2	1	3	----	176	31	0	0
New Hampshire	1	----	1	----	----	157	0	0
Vermont	----	----	----	----	41	62	0	0
Massachusetts	8	12	----	----	427	1,425	4	1
Rhode Island	----	----	----	----	518	17	1	0
Connecticut	2	3	5	1	1,493	126	0	0
Middle Atlantic States:								
New York	23	48	[1] 3	[1] 12	3,149	1,220	24	5
New Jersey	26	15	16	18	1,908	781	5	2
Pennsylvania	36	58	----	----	4,283	3,306	9	5
East North Central States:								
Ohio	16	26	6	6	1,808	1,559	6	7
Indiana	11	13	34	14	467	1,367	7	1
Illinois	47	31	26	51	2,322	2,418	29	13
Michigan	15	15	2	3	6,587	281	2	0
Wisconsin	3	2	32	33	1,727	2,030	1	1
West North Central States:								
Minnesota	7	8	1	2	597	302	2	1
Iowa	5	6	91	2	665	186	5	0
Missouri	29	35	31	49	528	608	14	0
North Dakota	2	5	5	2	30	165	1	0
South Dakota	4	3	2	----	67	425	0	0
Nebraska	5	11	2	----	373	369	2	2
Kansas	14	11	15	2	1,136	635	1	4
South Atlantic States:								
Delaware	1	4	----	----	4	108	0	0
Maryland [2]	8	2	4	4	77	2,597	9	0
District of Columbia	7	3	----	2	60	97	9	0
Virginia	13	7	----	----	509	1,139	7	6
West Virginia	10	20	35	----	390	97	11	0
North Carolina	12	12	21	25	341	2,174	0	2
South Carolina	2	8	142	324	29	443	0	0
Georgia [2]	2	6	----	----	----	252	0	0
Florida	4	4	5	----	28	911	0	1

See footnotes at end of table.

Cases of certain communicable diseases reported by telegraph by State health officers for weeks ended May 4, 1935, and May 5, 1934—Continued

Division and State	Diphtheria		Influenza		Measles		Meningococcus meningitis	
	Week ended May 4, 1935	Week ended May 5, 1934	Week ended May 4, 1935	Week ended May 5, 1934	Week ended May 4, 1935	Week ended May 5, 1934	Week ended May 4, 1935	Week ended May 5, 1934
East South Central States:								
Kentucky	10	11	9	8	450	509	0	2
Tennessee	13	9	35	47	41	526	7	3
Alabama	19	5	35	66	175	703	1	3
Mississippi [3]	5	8					0	1
West South Central States:								
Arkansas	2	9	16	9	60	38	3	2
Louisiana	19	22	1	5	70	196	0	0
Oklahoma [4]	1	3	60	42	194	310	0	6
Texas [3]	34	52	146	228	68	852	1	3
Mountain States:								
Montana [5]	6	5	41	40	445	108	1	0
Idaho		1			6	33	0	0
Wyoming [5]	2				27	130	0	0
Colorado [5]	5	9			247	691	1	0
New Mexico	6	4		1	31	180	0	0
Arizona	1		10	4	20	76	0	0
Utah [3]	1	1			7	166	0	0
Pacific States:								
Washington	2	2			439	240	3	0
Oregon [5]	4	3	22	19	264	79	2	0
California	25	44	48	49	1,595	930	7	1
Total	470	557	905	1,068	33,879	31,055	175	72
First 18 weeks of year	11,999	14,170	98,034	42,608	490,633	471,265	2,487	1,027

Division and State	Poliomyelitis		Scarlet fever		Smallpox		Typhoid fever	
	Week ended May 4, 1935	Week ended May 5, 1934	Week ended May 4, 1935	Week ended May 5, 1934	Week ended May 4, 1935	Week ended May 5, 1934	Week ended May 4, 1935	Week ended May 5, 1934
New England States:								
Maine	0	0	5	11	0	0	0	0
New Hampshire	0	0	23	8	0	0	0	0
Vermont	0	0	9	2	1	0	0	17
Massachusetts	1	1	210	217	0	0	4	1
Rhode Island	0	0	9	20	0	0	1	1
Connecticut	1	0	90	60	0	0	1	1
Middle Atlantic States:								
New York	0	4	961	768	0	0	3	7
New Jersey	0	0	164	177	0	0	3	3
Pennsylvania	0	0	590	642	0	0	8	9
East North Central States:								
Ohio	0	0	731	820	0	1	3	6
Indiana	0	0	131	159	0	5	0	7
Illinois	1	1	1,269	575	0	4	4	4
Michigan	1	1	331	672	1	1	5	8
Wisconsin	0	0	427	187	16	28	0	3
West North Central States:								
Minnesota	2	0	413	57	5	12	0	0
Iowa	0	0	91	75	0	11	0	0
Missouri	1	0	64	87	0	4	0	8
North Dakota	0	0	126	21	0	0	0	0
South Dakota	0	1	13	13	11	3	0	1
Nebraska	0	0	57	35	35	9	1	0
Kansas	0	0	75	43	36	5	3	3
South Atlantic States:								
Delaware	0	0	5	4	0	0	1	1
Maryland [3]	1	1	123	52	0	0	1	9
District of Columbia	0	0	78	10	0	0	0	0
Virginia	1	0	36	34	0	0	5	7

See footnotes at end of table.

Cases of certain communicable diseases reported by telegraph by State health officers for weeks ended May 4, 1935, and May 5, 1934—Continued

Division and State	Poliomyelitis		Scarlet fever		Smallpox		Typhoid fever	
	Week ended May 4, 1935	Week ended May 5, 1934	Week ended May 4, 1935	Week ended May 5, 1934	Week ended May 4, 1935	Week ended May 5, 1934	Week ended May 4, 1935	Week ended May 5, 1934
South Atlantic States—Continued								
West Virginia	0	0	64	77	0	1	5	5
North Carolina	2	1	9	19	0	3	4	2
South Carolina	0	0	7	5	2	0	4	3
Georgia [2]	0	1	-------	2	0	0	11	4
Florida	0	0	4	3	0	0	5	3
East South Central States:								
Kentucky	0	0	33	57	1	0	15	8
Tennessee	1	0	19	29	0	0	2	7
Alabama	0	0	6	8	0	0	3	6
Mississippi [2]	0	1	7	7	0	0	5	1
West South Central States:								
Arkansas	1	1	2	7	2	1	2	7
Louisiana	2	0	17	11	0	0	15	18
Oklahoma [4]	0	0	13	15	3	3	6	3
Texas [3]	0	2	39	52	7	27	5	18
Mountain States:								
Montana [5]	0	0	10	10	4	2	0	1
Idaho	0	3	4	3	0	0	0	2
Wyoming [5]	0	0	37	11	17	1	0	0
Colorado [5]	0	0	251	27	5	5	0	0
New Mexico	1	0	10	12	6	0	6	4
Arizona	0	2	51	10	0	0	1	0
Utah [2]	0	0	129	10	0	0	0	1
Pacific States:								
Washington	2	0	61	49	57	8	1	5
Oregon [5]	0	1	-------	42	9	2	1	2
California	3	13	199	201	21	11	4	5
Total	21	34	7,003	5,426	239	147	138	201
First 18 weeks of year	430	382	129,474	108,440	3,457	2,714	2,394	2,829

[1] New York City only.
[2] Week ended earlier than Saturday.
[3] Typhus fever, week ended May 4, 1935, 7 cases, as follows: Georgia, 4; Texas, 3.
[4] Exclusive of Oklahoma City and Tulsa.
[5] Rocky Mountain spotted fever, week ended May 4, 1935, 9 cases, as follows: Montana, 3; Wyoming, 2; Colorado, 2; Oregon, 2.

SUMMARY OF MONTHLY REPORTS FROM STATES

The following summary of cases reported monthly by States is published weekly and covers only those States from which reports are received during the current week.

State	Meningococcus meningitis	Diphtheria	Influenza	Malaria	Measles	Pellagra	Poliomyelitis	Scarlet fever	Smallpox	Typhoid fever
March 1935										
Nevada	3	-------	16	-------	41	-------	0	20	0	0
Puerto Rico	-------	62	63	846	163	-------	1	-------	0	44
Virginia	23	77	2,417	4	5,527	10	1	273	1	9
April 1935										
Connecticut	3	17	28	-------	6,402	-------	1	473	0	1
Delaware	-------	2	3	-------	56	-------	0	54	0	0
Missouri	36	143	403	26	3,324	-------	2	297	8	16

March 1935

	Cases
Chicken pox:	
Nevada	96
Puerto Rico	159
Virginia	529
Diarrhea and dysentery:	
Virginia	66
Dysentery:	
Puerto Rico	25
Virginia (amoebic)	2
Filariasis:	
Puerto Rico	1
Leprosy:	
Puerto Rico	1
Mumps:	
Nevada	1
Puerto Rico	90
Virginia	412
Ophthalmia neonatorum:	
Puerto Rico	10
Virginia	1
Puerperal septicemia:	
Puerto Rico	6
Rocky Mountain Spotted fever:	
Nevada	1
Septic sore throat:	
Nevada	1
Virginia	5
Tetanus:	
Puerto Rico	9
Virginia	1

March 1935—Continued

	Cases
Tetanus, infantile:	
Puerto Rico	4
Trachoma:	
Puerto Rico	1
Virginia	1
Tularaemia:	
Virginia	1
Undulant fever:	
Virginia	5
Whooping cough:	
Nevada	56
Puerto Rico	352
Virginia	451

April 1935

Chicken pox:	
Connecticut	558
Delaware	39
Missouri	369
Conjunctivitis:	
Connecticut	25
Dysentery:	
Connecticut (amoebic)	1
Missouri	11
Epidemic encephalitis:	
Missouri	1
German measles:	
Connecticut	677
Delaware	2

April 1935—Continued

	Cases
Lead poisoning:	
Connecticut	1
Mumps:	
Connecticut	322
Delaware	54
Missouri	475
Ophthalmia neonatorum:	
Connecticut	1
Missouri	1
Rabies in animals:	
Connecticut	7
Missouri	15
Septic sore throat:	
Connecticut	38
Missouri	61
Tetanus:	
Connecticut	2
Trachoma:	
Missouri	3
Trichinosis:	
Connecticut	2
Undulant fever:	
Connecticut	5
Missouri	6
Whooping cough:	
Connecticut	212
Delaware	7
Missouri	266

WEEKLY REPORTS FROM CITIES

City reports for week ended Apr. 27, 1935

This table summarizes the reports received regularly from a selected list of 121 cities for the purpose of showing a cross section of the current urban incidence of the communicable diseases listed in the table. Weekly reports are received from about 700 cities, from which the data are tabulated and filed for reference]

State and city	Diphtheria, cases	Influenza		Measles, cases	Pneumonia, deaths	Scarlet fever, cases	Smallpox, cases	Tuberculosis, deaths	Typhoid fever, cases	Whooping cough, cases	Deaths, all causes
		Cases	Deaths								
Maine:											
Portland	0		0	1	7	0	0	0	0	0	25
New Hampshire:											
Concord	1		2	0	1	1	0	0	0	0	13
Nashua	0			0		0	0		0	0	
Vermont:											
Barre	0		0	1	1	1	0	1	0	4	3
Burlington	0			16		4	0		0	0	6
Massachusetts:											
Boston	0		1	41	10	42	0	10	0	18	257
Fall River	0		0	5	2	11	0	3	0	5	26
Springfield	0		0	113	5	13	0	4	0	5	49
Worcester	0		0	4	12	18	0	3	1	9	58
Rhode Island:											
Pawtucket	0		0	2	0	0	0	0	0	0	16
Providence	0		0	288	9	7	0	4	0	2	60
Connecticut:											
Bridgeport	0		0	6	6	13	0	0	0	2	37
Hartford	1		0	35	5	10	0	0	0	12	50
New Haven	0		0	481	1	2	0	0	0	0	47
New York:											
Buffalo	1		1	50	15	62	0	5	0	28	152
New York	20	5	5	1,585	160	641	0	106	0	208	1,653
Rochester	0		0	210	6	25	0	0	0	16	78
Syracuse	0		0	458	2	13	0	1	0	15	42
New Jersey:											
Camden	1		0	1	2	17	0	0	0	3	33
Newark	1	4	1	646	11	7	0	7	0	55	103
Trenton	0		0	7	3	11	0	3	0	0	44
Pennsylvania:											
Philadelphia	4	11	5	66	47	122	0	39	0	87	590
Pittsburgh	0	3	1	510	29	52	0	11	0	17	191
Reading	1		0	84	2	2	0	0	0	0	23
Scranton	0			27		3	0		0	0	

City reports for week ended Apr. 27, 1935—Continued

State and city	Diphtheria, cases	Influenza		Measles, cases	Pneumonia, deaths	Scarlet fever, cases	Smallpox, cases	Tuberculosis, deaths	Typhoid fever, cases	Whooping cough, cases	Deaths, all causes
		Cases	Deaths								
Ohio:											
Cincinnati	4		3	10	11	21	0	9	0	1	149
Cleveland	6	33	2	530	21	66	0	12	0	36	206
Columbus	2	2	2	146	5	18	0	2	1	4	96
Toledo	0	1	1	144	2	11	0	2	0	10	54
Indiana:											
Fort Wayne	2		0	8	1	3	0	0	0	1	25
Indianapolis	0		1	158	17	14	0	3	0	25	107
South Bend	0		0	1	1	8	0	0	0	0	14
Terre Haute	0		0	0	0	1	0	0	0	0	20
Illinois:											
Chicago	27	7	7	1,549	52	716	0	34	1	65	712
Springfield	1	1	0	24	6	19	0	3	0	11	27
Michigan:											
Detroit	6	2	1	2,503	42	103	0	13	0	97	290
Flint	1		0	19	7	16	0	0	0	2	33
Grand Rapids	0		1	196	4	18	0	2	0	15	42
Wisconsin:											
Kenosha	0		0	42	1	22	0	0	0	2	5
Milwaukee	1	1	1	234	4	90	0	4	0	27	99
Racine	0		0	114	0	9	0	1	0	19	19
Superior	0		0	28	0	2	0	1	0	2	12
Minnesota:											
Duluth	0		0	184	0	8	0	2	0	2	29
Minneapolis	0		0	189	9	203	0	1	0	19	106
St. Paul	0		0	34	7	100	0	0	0	12	63
Iowa:											
Davenport	0			0		1	0		0	0	
Des Moines	0			76		3	0		0	0	36
Sioux City	2			2		2	0		0	3	
Waterloo				1		7	0		0	3	
Missouri:											
Kansas City	3		0	96	7	12	0	2	0	4	82
St. Joseph	2		0	4	4	2	0	3	0	1	34
St. Louis	18		1	35	17	26	0	12	3	19	246
North Dakota:											
Fargo	0		0	6	2	7	0	1	0	2	7
Grand Forks	0			0		1	0		0	0	
South Dakota:											
Aberdeen	0			25		1	0		0	0	
Sioux Falls	0			0		0	0		0	0	10
Nebraska:											
Omaha	2		0	79	8	4	4	5	0	2	66
Kansas:											
Topeka											
Wichita	0		0	150	2	1	2	0	0	4	23
Delaware:											
Wilmington	0		0	11	10	1	0	2	0	0	45
Maryland:											
Baltimore	2	1	0	27	20	47	0	14	2	23	230
Cumberland	1		0	2	0	2	0	1	0	0	14
Frederick	0	2	0	1	1	3	0	0	0	0	7
Dist. of Columbia:											
Washington	9	1	1	56	20	64	0	15	1	6	188
Virginia:											
Lynchburg	0		0	10	0	1	0	0	0	18	12
Norfolk	1		0	34	6	1	0	0	0	1	36
Richmond	0		0	76	2	0	0	3	0	0	45
Roanoke	0		0	19	1	0	0	0	0	3	15
West Virginia:											
Charleston	1		0	12	1	0	0	0	0	5	8
Huntington	0			0		0	0		0	0	
Wheeling	0		0	86	5	15	0	1	0	0	19
North Carolina:											
Raleigh	0		0	3	3	0	0	0	0	8	18
Wilmington	0		0	3	0	0	0	0	0	5	8
Winston-Salem	1		0	0	1	0	0	1	0	13	12
South Carolina:											
Charleston	0	10	0	0	4	1	0	1	0	1	20
Columbia											
Greenville	0					0	0		0	3	
Georgia:											
Atlanta	3	10	2	2	8	2	0	7	0	4	80
Brunswick	0		0	0	0	0	0	0	0	4	3
Savannah	0		2	0	2	0	0	6	0	0	32

City reports for week ended Apr. 27, 1935—Continued

State and city	Diphtheria, cases	Influenza		Measles, cases	Pneumonia, deaths	Scarlet fever, cases	Smallpox, cases	Tuberculosis, deaths	Typhoid fever, cases	Whooping cough, cases	Deaths, all causes
		Cases	Deaths								
Florida:											
Miami	1	1	1	4	0	1	0	2	0	4	31
Tampa	0	2	2	53	1	1	0	0	0	2	30
Kentucky:											
Ashland	0	----	----	6	----	0	0	----	1	0	----
Lexington	0	----	----	5	2	1	0	2	0	5	20
Louisville	4	----	0	247	11	28	0	4	1	13	91
Tennessee:											
Memphis	2	----	1	2	8	2	0	3	0	19	88
Nashville	1	----	0	1	0	3	0	4	0	4	50
Alabama:											
Birmingham	0	5	0	22	6	1	0	3	0	4	53
Mobile	2	----	0	7	2	0	0	0	0	----	29
Montgomery	1	----	----	13	----	0	0	----	0	1	----
Arkansas:											
Fort Smith	----	----	----	----	----	----	----	----	----	----	----
Little Rock	0	----	2	27	8	0	0	4	0	12	16
Louisiana:											
New Orleans	17	6	1	33	15	8	0	11	0	0	162
Shreveport	0	----	0	2	5	0	0	4	1	0	30
Oklahoma:											
Tulsa	0	----	----	2	----	0	0	----	0	13	----
Texas:											
Dallas	5	2	2	0	7	1	0	3	0	8	50
Fort Worth	0	----	0	----	7	4	0	1	0	0	45
Galveston	0	----	0	0	1	0	0	2	0	0	25
Houston	5	----	1	3	4	0	3	3	0	0	59
San Antonio	4	----	1	0	4	1	0	6	0	0	52
Montana:											
Billings	1	----	----	11	----	0	0	----	0	0	6
Great Falls	0	----	0	12	3	0	0	0	0	17	9
Helena	0	----	0	10	0	0	0	0	0	2	3
Missoula	0	----	0	15	0	0	0	0	0	0	3
Idaho:											
Boise	----	----	----	----	----	----	----	----	----	----	----
Colorado:											
Denver	5	43	2	167	3	121	0	5	0	6	73
Pueblo	0	----	0	94	1	5	0	1	0	12	13
New Mexico:											
Albuquerque	1	----	0	8	1	1	0	1	0	1	7
Utah:											
Salt Lake City	0	----	0	2	2	131	0	0	0	120	24
Nevada:											
Reno	0	----	0	0	0	0	0	0	0	0	3
Washington:											
Seattle	0	----	1	245	6	8	0	5	0	7	95
Spokane	0	1	1	118	5	8	0	0	0	3	29
Tacoma	1	----	1	1	4	0	0	0	0	1	26
Oregon:											
Portland	0	----	0	113	7	13	0	0	0	1	94
Salem	0	----	----	2	----	1	0	----	0	0	----
California:											
Los Angeles	13	26	2	71	19	42	2	25	0	19	313
Sacramento	1	----	0	250	2	7	0	2	0	1	32
San Francisco	0	2	0	56	9	11	0	10	1	23	160

City reports for week ended Apr. 27, 1935—Continued

State and city	Meningococcus meningitis		Poliomyelitis cases
	Cases	Deaths	
Massachusetts:			
Boston	1	0	0
Fall River	1	1	0
Rhode Island:			
Providence	1	0	0
New York:			
New York	22	6	0
Pennsylvania:			
Philadelphia	3	2	0
Pittsburgh	1	2	0
Ohio:			
Cincinnati	13	5	0
Cleveland	3	2	0
Toledo	1	1	0
Indiana:			
Indianapolis	1	0	0
Terre Haute	1	0	0
Illinois:			
Chicago	11	1	0
Michigan:			
Detroit	2	0	0
Wisconsin:			
Milwaukee	1	1	0
Iowa:			
Sioux City	2		0
Missouri:			
Kansas City	0	1	0
St. Joseph	2	1	0
St. Louis	2	1	0
Maryland:			
Baltimore	6	1	0
Cumberland	1	0	0
District of Columbia:			
Washington	4	1	0
Virginia:			
Lynchburg	1	0	0
Florida:			
Miami	0	1	0
Kentucky:			
Ashland	1	0	0
Lexington	1	1	0
Louisville	5	0	0
Tennessee:			
Memphis	1	1	0
Nashville	1	0	0
Alabama:			
Montgomery	1		0
Arkansas:			
Little Rock	0	1	0
Louisiana:			
New Orleans	0	0	1
Oklahoma:			
Tulsa	1	1	0
Washington:			
Seattle	0	1	0
Spokane	2	1	0
Tacoma	1	1	0
Oregon:			
Portland	1	2	0
California:			
Los Angeles	1	0	0
San Francisco	0	0	1

Epidemic encephalitis.—Cases: New York, 2; Newark, N. J., 1; Toledo, 1; Chicago, 1; Kansas City, Mo., 1; Louisville, 1. Instead of 15 cases of epidemic encephalitis, 1 case should have been published in the Public Health Reports of Apr. 12, 1935, p. 533, as occurring at Louisville, Ky., during the week ended Mar. 23.

Pellagra.—Cases: Boston, 2; Savannah, 1; New Orleans, 2; Los Angeles, 1; San Francisco, 1.

Typhus fever.—Cases: New York, 1; Savannah, 1; Miami, 1.

FOREIGN AND INSULAR

ITALY

Communicable diseases—4 weeks ended February 3, 1935.—During the 4 weeks ended February 3, 1935, cases of certain communicable diseases were reported in Italy, as follows:

Disease	Jan. 7-13		Jan. 14-20		Jan. 21-27		Jan. 28-Feb. 3	
	Cases	Communes affected	Cases	Communes affected	Cases	Communes affected	Cases	Communes affected
Anthrax	10	10	10	9	12	11	9	9
Cerebrospinal meningitis	10	9	11	10	11	8	6	6
Chicken pox	454	131	285	89	348	110	362	123
Diphtheria and croup	528	285	563	283	483	264	497	259
Dysentery	6	5	4	4	3	3	1	1
Lethargic encephalitis					1	1		
Measles	1,936	305	1,963	257	2,105	307	2,209	333
Poliomyelitis	3	3	5	5	6	6	4	4
Scarlet fever	303	123	249	96	333	117	217	108
Typhoid fever	315	168	299	158	239	143	206	142

PANAMA CANAL ZONE

Communicable diseases—January–March 1935.—During the months of January, February, and March 1935, certain communicable diseases, including imported cases, were reported in the Panama Canal Zone and terminal cities, as follows:

Disease	January		February		March	
	Cases	Deaths	Cases	Deaths	Cases	Deaths
Chicken pox	10		2		17	
Diphtheria	9		10	1	4	
Dysentery (amoebic)	25		21		18	2
Dysentery (bacillary)				2	2	2
Leprosy					1	
Malaria	137	4	177	1	89	5
Measles	2		1		4	
Mumps	4				1	
Paratyphoid fever			1			
Pneumonia		17		18		19
Relapsing fever					1	
Tuberculosis		31		26		26
Typhoid fever	1		3			
Typhus fever	1	1	1			
Whooping cough	8	1		1	3	

CHOLERA, PLAGUE, SMALLPOX, TYPHUS FEVER, AND YELLOW FEVER

(NOTE.—A table giving current information of the world prevalence of quarantinable diseases appeared in the PUBLIC HEALTH REPORTS for Apr. 26, 1935, pp. 580-594. A similar cumulative table will appear in the PUBLIC HEALTH REPORTS to be issued May 31, 1935, and thereafter, at least for the time being, in the issue published on the last Friday of each month.)

Plague

Hawaii Territory—Hawaii Island—Hamakua District—Pohakea.—On April 24, 1935, 1 plague-infected rat was reported at Pohakea, Hamakua District, Island of Hawaii, Hawaii Territory.

Senegal—Thies.—During the period April 11–20, 1935, 1 case of plague with 1 death was reported at Thies, Senegal.

United States—California.—A report of 7 plague-infected ground squirrels in Modoc County, California, will be found on page 657 of the PUBLIC HEALTH REPORTS for May 10, 1935.