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THE SPECIFICITY OF THE COMPLEMENT FIXATION TEST IN ENDEMIC TYPHUS FEVER USING A RICKETTSIAL ANTIGEN¹

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It was reported recently (1) that the complement fixation test could be used in determining past infection with endemic typhus fever, as well as recent infection, using a rickettsial antigen. The specificity of the test was studied in relation to other rickettsial diseases, including Rocky Mountain spotted fever, "Q" fever, and European typhus. In this report the specificity of the test is further considered; serums have been tested from more cases of endemic typhus fever and from cases of other diseases.

The endemic typhus serums were from cases which occurred in Alabama and Georgia, and the strain had been isolated and proved in 15 of the cases. In 37 others the diagnosis was based on clinical symptoms.

Other diseases from which serums were obtained include tuberculosis, leprosy, malaria, syphilis, lymphopathia venereum, typhoid fever, amebiasis, trachoma, rheumatic fever, undulant fever, and tularemia. Patients from whom many of the specimens were obtained were in the marine hospitals in Norfolk, Va., and Baltimore, Md., the National Leprosarium in Carville, La., St. Elizabeths Hospital in Washington, D. C., and the trachoma hospitals in Richmond, Ky., and Rolla, Mo. The serums from these cases were from freshly drawn blood. In addition, tests were made on a number of serums which had been received at the National Institute of Health for agglutination tests. Some of these were of recent origin, but most of them had been stored at refrigerator temperature for periods of 1 to 24 months. Among these were serums from cases of undulant fever, tularemia, and rheumatic fever. Typhoid serums were obtained from the Hygienic Laboratory of the Arkansas State Board of Health.

Procedure.—The antigen was prepared from yolk sacs infected with the wild rat strain of endemic typhus fever referred to in the previous publication (1). After several passages of the virus by inoculation into the yolk of 6-day chick embryos, the yolk sacs were very heavily

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infected. These were macerated after draining to free from excess yolk, and suspended in 0.85 percent salt solution containing 1:10,000 merthiolate to a concentration of 10 percent. The suspension was centrifuged lightly to precipitate the large particles. The supernatant fluid was then centrifuged in an angle centrifuge at 4,000 revolutions per minute for 1 hour. The precipitate was removed and suspended in 0.85 percent sterile saline with merthiolate, to the original volume. The suspensions of rickettsiae remained in the refrigerator for periods varying from 1 to several weeks, during which time more tissue precipitated and the rickettsiae remained in suspension. Titrations of the antigen with known human and guinea pig serums showed 4+ fixation in dilution of 1:8, and this dilution was used in all tests.

The test was carried out as previously described (1), 0.2 cc. amounts of inactivated serum in dilutions ranging from 1:2 to 1:64 or higher, 0.2 cc. amounts of antigen, and 0.2 cc. amounts of complement being mixed and incubated for 1 hour at 37° C., after which 0.4 cc. of sensitized sheep cells were added and incubation continued for another hour.

Results.—The results obtained with the typhus serums are shown in table 1. The complement fixation and the Weil-Felix titers are shown in parallel columns. Fixation to 3+ or 4+ in the dilutions indicated was considered as the titer of the serum. Likewise, in the Weil-Felix test agglutination to 3+ or 4+ in the dilutions indicated was considered as the titer of the serums tested. The date of illness and the method of diagnosis are shown. Table 2 summarizes the results obtained with all the other serums tested.

DISCUSSION

Among the 15 proved cases of endemic typhus all serums were positive in dilutions of 1:4 to 1:1,024. These were cases in which typhus fever had occurred from 2 months to 6 years previously. Fourteen were positive in dilutions of 1:8 or over, while 1 was positive in a dilution of 1:4. The 2 cases which were of the most recent origin (2 months) were positive in dilutions of 1:512 and 1:1,024, while the one of earliest origin (67 months) had a titer of 1:16. The 3 with the lowest titers (1:4 and 1:8) had had the disease 23 months, 36 months, and 39 months previously.

There were 37 serums from cases diagnosed as endemic typhus fever from the clinical symptoms, although a few of these were cases which had not actually been reported as typhus fever. All of the patients had had the disease in 1940 with the exception of No. 41 who had had it 3 years previously. Thirty-two of the serums had titers ranging from 1:8 to 1:1,024. Five serums had titers below 1:8 (2 had a titer of 1:2 and 3 had 1:4). It is of interest that such a high percentage of the cases were correctly diagnosed as indicated by the results of the complement fixation test, although it is to be considered that the cases

occurred in an area where the disease is endemic. Possibly some of those with the low titers were incorrectly diagnosed.

There is a certain correlation between the titer of the serum and the length of time elapsing between the date of illness and the time of testing the serum, higher titers being evident, in general, during the first few months after illness, although the irregularities suggest some relationship of the titer to the severity of the illness.

TABLE 1.—*Typhus serums*

Serum No.	Patient's name	Date of typhus	Status of diagnosis	Complement fixation titer	Weil-Felix titer (X ₁₀)
1.	W. D. C.	Sept. 14, 1935	Strain isolated	1:16	1:10
2.	G. S.	Dec. 9, 1936	do.	1:32	1:40
3.	L. J.	May 18, 1937	do.	1:32	1:20
4.	R. H.	Sept. 25, 1939	do.	1:128	1:20
5.	R. W.	June 19, 1939	do.	1:64	1:40
6.	J. B. C.	Oct. 11, 1940	do.	1:256	1:20
7.	R. R.	July 13, 1938	do.	1:8	1:20
8.	J. H. W.	Apr. 21, 1938	do.	1:4	1:10
9.	D. W. R.	Sept. 10, 1938	do.	1:32	1:20
10.	E. W.	June 11, 1940	do.	1:64	1:20
11.	J. B.	Feb. 7, 1939	do.	1:64	1:20
12.	T. L. J.	May 20, 1939	do.	1:8	1:160
13.	G. W. B.	June 20, 1940	do.	1:64	0
14.	D. B.	Jan. 27, 1940	Clinical diagnosis	1:16	1:10
15.	E. S. M.	June 8, 1940	do.	1:4	1:40
16.	H. W. S.	Jan. 20, 1940	do.	1:128	1:80
17.	W. R. Y.	Nov. 2, 1940	do.	1:256	1:160
18.	A. G.	Oct. 27, 1940	do.	1:64	1:20
19.	R. B.	Aug. 24, 1940	do.	1:64	1:10
20.	M. K.	Oct. 26, 1940	do.	1:256	1:1,280
21.	M. M.	July 20, 1940	do.	1:256	1:80
22.	H. E.	June 1, 1940	do.	1:256	1:20
23.	M. D.	Nov. 30, 1940	do.	1:256	1:10
24.	R. R.	Aug. 24, 1940	do.	1:64	1:40
25.	M. A.	Sept. 14, 1940	do.	1:128	1:160
26.	M. R.	Nov. 16, 1940	do.	1:8	1:80
27.	S. B.	Nov. 9, 1940	do.	1:32	1:80
28.	Mrs. W. S.	Nov. 2, 1940	do.	1:256	1:80
29.	J. J.	Jan. 13, 1940	do.	1:64	1:20
30.	T. C.	Aug. 3, 1940	do.	1:64	1:20 (2+)
31.	J. B. D.	Sept. 21, 1940	do.	1:32	1:20 (2+)
32.	G. G.	Jan. 6, 1940	do.	1:64	1:80
33.	M. J.	Oct. 12, 1940	do.	1:256	1:20
34.	E. L.	Nov. 9, 1940	do.	1:256	1:40
35.	Mrs. E. B. S.	Oct. 12, 1940	do.	1:4	1:160
36.	M. S.	Sept. 21, 1940	do.	1:64	1:10 (2+)
38.	A. A.	Sept. 28, 1940	do.	1:32	1:40
39.	Mrs. A. S.	1940, not reported	do.	1:128	1:10
40.	T. M. A.	1940, not reported	do.	1:256	1:40
41.	J. D. A.	3 years ago, reported (?)	do.	1:8	1:10 (2+)
42.	R. W.	1940, not reported (?)	do.	1:64	1:160
43.	F. E. G.	Nov. 9, 1940	do.	1:128	1:320
44.	Mrs. E. R.	Nov. 30, 1940	do.	1:16	1:320
45.	Mrs. P. L.	Oct. 27, 1940	do.	1:256	1:20
46.	Mrs. A. H.	July 20, 1940	do.	1:2	1:10 (2+)
47.	E. G. P.	Feb. 8, 1941	Strain isolated	1:1,024	1:1,280
48.	W. B. G.	Feb. 8, 1941	do.	1:512	1:40
49.	E. B. W.	July 27, 1940	Clinical diagnosis	1:64	1:40
50.	Mrs. A. A. M.	1940, not reported	do.	1:2	1:10
51.	Dr. W. A. M.	do.	do.	1:4	1:10
52.	R. T.	Nov. 6, 1940	do.	1:256	1:320
53.	W. A. S.	1940	do.	1:64	1:10 (2+)

The Weil-Felix titer of the serums from most of the cases which had occurred a year or more prior to the date of obtaining the blood for the test had decreased to a low point (1:40 or lower). In only 2 cases was the titer higher than 1:40 (No. 12, 25 months, titer 1:160, and No. 32, 15 months, titer 1:80). The corresponding complement

fixation titers were 1:8 and 1:64. Also, many of the serums obtained less than a year after illness had low Weil-Felix titers (e. g., No. 6, 6 months after illness, complement fixation titer 1:256, Weil-Felix titer 1:20; No. 13, 10 months after illness, complement fixation titer 1:64, Weil-Felix titer 0). On the other hand, there was a certain correlation in a number of the serums in this group (e. g., No. 47, 2 months after illness, complement fixation titer 1:1,024, Weil-Felix titer 1:1,280; No. 20, 6 months after illness, complement fixation titer 1:256, Weil-Felix titer 1:1,280; No. 15, 10 months after illness, complement fixation titer 1:4, Weil-Felix titer 1:40). More detailed studies are suggested to determine further the relationship of the complement fixation to the Weil-Felix test.

TABLE 2.—*Serums from other diseases*

Number of specimens	Disease	Complement fixation	Remarks
14	Tuberculosis.....	0.....	
10	Leprosy.....	0 to very slight.....	7 fixed complement in dilution 1:2 (1+ or 2+).
6	Malaria.....	0.....	2 cases active; 2 cases cured; 2 cases with tabes dorsalis.
10	Syphilis.....	0.....	3 cases primary; 3 cases secondary; 4 cases tertiary.
10	Rheumatic fever.....	0.....	
7	Undulant fever.....	0 to very slight.....	6 fixed complement in dilutions 1:2 to 1:4 (1+ or 2+).
			Titers against abortus antigen were 1:160 to 1:5120.
13	Tularemia.....	0 to very slight.....	7 fixed complement in dilutions 1:2 to 1:8 (1+ or 2+).
			Titers against tularensis antigen were 1:8 to 1:1280.
8	Typhoid fever.....	0.....	
9	Trachoma.....	0.....	3 cases, stage IIa; 2 cases, stage IIb; 3 cases, stage III;
2	Lymphopathia venereum.	0.....	1 case, stage IV.
1	Psittacosis.....	0.....	
2	Amebiasis.....	0.....	

The results obtained with serums from patients with other diseases point to rather definite specificity of the test. Seven of the 10 leprosy serums tested showed slight fixation in the 1:2 dilution, which is probably of no significance. Serums from patients infected with the tubercle bacillus, another acid-fast organism, were completely negative.

Serums from 10 syphilis cases, including primary, secondary, and tertiary cases, were all negative. Among the virus diseases 2 specimens from lymphopathia venereum, 1 from psittacosis, and 9 from trachoma were all negative. Serums from 2 cases of amebiasis were negative. Specimens from 6 cases of malaria, 2 active, 2 cured, and 2 with tabes dorsalis, all gave negative results.

The 7 specimens from undulant fever and the 13 specimens from tularemia which gave positive readings with abortus and tularensis antigens in the agglutination test and which had been stored in the refrigerator for periods up to 24 months were negative in the complement fixation test except that some fixation was obtained in low dilutions (1:4 and once 1:8), but this fixation was never more complete than 2+. A number of these serums were slightly anticomplementary, probably owing to the considerable period of storage in some cases.

SUMMARY

The complement fixation test for endemic typhus fever has been shown to be specific by comparing the results obtained using serums from known proved cases of endemic typhus fever and from cases diagnosed clinically as endemic typhus with those obtained using serums from cases of syphilis, leprosy, tuberculosis, rheumatic fever, malaria, undulant fever, tularemia, trachoma, and a few specimens from miscellaneous diseases including lymphopathia venereum, psittacosis, and amebiasis.

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REFERENCE

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STUDIES OF SEWAGE PURIFICATION ¹XIV. THE ROLE OF *SPHAEROTILUS NATANS* IN ACTIVATED SLUDGE BULKING

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INTRODUCTION

The bulking of activated sludge has received the attention of sanitary engineers and chemists ever since the development of the process to large scale operation. Bulking has been considered the result of several different causes. In a recent excellent paper Heulekian and Ingols (1) divide bulking into two general classes—carbohydrate bulking and sewage bulking. They studied seven

¹ Previous articles in this series are:

Therhault, E. J., and McNamee, P. D.: Studies of sewage purification. I. Apparatus for the determination of dissolved oxygen in sludge-sewage mixtures. Pub. Health Rep., 50: 480 (1935). (Reprint No. 1680.)

Butterfield, C. T.: Studies of sewage purification. II. A zoogel-forming bacterium isolated from activated sludge. Pub. Health Rep., 50: 671 (1935). (Reprint No. 1696.)

Therhault, E. J.: Studies of sewage purification. III. The clarification of sewage. A review. Sewage Works J., 8: 377 (1935). Also, Pub. Health Rep., 50: 1581 (1935). (Reprint No. 1715.)

Smith, Russell S., and Purdy, W. C.: Studies of sewage purification. IV. The use of chlorine for the correction of sludge bulking in the activated sludge process. Sewage Works J., 8: 223-230 (1936). Pub. Health Rep., 51: 617 (1936). (Reprint No. 1746.)

McNamee, P. D.: Studies of sewage purification. V. Oxidation of sewage by activated sludge. Sewage Works J., 8: 562 (1936). Pub. Health Rep., 51: 1034 (1936). (Reprint No. 1774.)

Butterfield, C. T., Ruchhoff, C. C., and McNamee, P. D.: Studies of sewage purification. VI. Biochemical oxidation by sludges developed by pure cultures of bacteria isolated from activated sludge. Sewage Works J., 9: 173 (1937). Pub. Health Rep., 52: 287 (1937). (Reprint No. 1812.)

Footnote 1 continued on p. 1728.

factors that were involved, including oxygen supply, food concentration, sludge concentration, sludge condition, carbon to nitrogen ratio, temperature, and nitrates. Bulking, they said, was induced by an excessive development of sludge or certain organisms comprising the sludge, due to the improper balance of food in relation to sludge. These authors stressed aeration rate as an important factor in this phenomenon.

One variety of bulking is commonly associated with excessive growths of *Sphaerotilus natans*. When this type of bulking occurs, carbohydrates are often found in the sewage influent. Lackey and Wattie (2), in a previous paper of this series, reviewed instances of activated sludge bulking in which *Sphaerotilus natans* was considered the causative agent, and presented the biology of this organism. The limits of nutrient elements requisite for the growth of *Sphaerotilus natans* were determined, and in an extensive search no substance was found, common or apt to occur in sewage, which stimulated the organism to excessive growth. These investigators found *Sphaerotilus natans* to be a strict aerobe. Littman (3) has contributed a study of the carbon and nitrogen transformations of sewage by *Sphaerotilus*. He found that a concentration of 757 p. p. m. of *Sphaerotilus*, dosed with sterile sewage and aerated, removed a maximum of 56 percent of the 5-day B. O. D. of the sewage after 4 hours and also determined the carbon dioxide produced. He concluded that the *Sphaerotilus* sludge produced had high sludge indices, exerted a moderate purifying action on sewage, and that certain types of bulking appeared to be the result of the overgrowth of activated sludge by these organisms.

The view that carbohydrates are specific stimulants in inducing bulking is quite common. Ingols and Heukelekian (4) expressed the view that glucose stimulates *Sphaerotilus natans* to a greater extent than zooglyphic bacteria even under aerobic conditions. Ingols

(Footnote 1 continued from page 1727)

Ruchhoft, C. C., McNamee, P. D., and Butterfield, C. T.: Studies of sewage purification. VII. Biochemical oxidation by activated sludge. *Sewage Works J.*, 10: 661 (1938). *Pub. Health Rep.*, 53: 1690-1718 (1938). (Reprint No. 1987.)

Butterfield, C. T., and Wattie, Elsie: Studies of sewage purification. VIII. Observations on the effect of variations in the initial numbers of bacteria and of the dispersion of sludge flocs on the course of oxidation of organic material by bacteria in pure culture. *Pub. Health Rep.*, 53: 1912 (1938). (Reprint No. 1999.)

Ruchhoft, C. C., Butterfield, C. T., McNamee, P. D., and Wattie, Elsie: Studies of sewage purification. IX. Total purification, oxidation, adsorption, and synthesis of nutrient substrates by activated sludge. *Sewage Works J.*, 11: 195 (1939). *Pub. Health Rep.*, 54: 468 (1939). (Reprint No. 2040.)

Ruchhoft, C. C., and Smith, R. S.: Studies of sewage purification. X. Changes in characteristics of activated sludge induced by variations in applied load. *Sewage Works J.*, 11: 409 (1939). *Pub. Health Rep.*, 54: 924 (1939). (Reprint No. 2074.)

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Ruchhoft, C. C., Kachmar, J. F., and Placak, O. R.: Studies of sewage purification. XII. Metabolism of glucose by activated sludge. *Pub. Health Rep.*, 55: 582-601 (1940). (Reprint No. 2149.)

Lackey, James B., and Wattie, Elsie: Studies of sewage purification. XIII. The biology of *Sphaerotilus natans* Kutzling in relation to bulking of activated sludge. *Pub. Health Rep.*, 55: 975-988 (1940). (Reprint No. 2166.)

(5) not only considers *Sphaerotilus natans* as a facultative anaerobe but concludes that *Sphaerotilus natans* grows much more rapidly with less oxygen. The physiology of both *Sphaerotilus natans* and zooglear bacteria should be very carefully studied so that our understanding of the causes and cure for *Sphaerotilus* overgrowths and bulking difficulties will be sound. In this paper, therefore, we have studied the growth and metabolic response of *Sphaerotilus natans* to carbohydrates under aerobic and anaerobic conditions. While to some this may seem far removed from the immediate problem of bulking, such pure culture information seems imperative for a complete understanding of the bulking phenomenon. Following this a series of experiments was performed in which bulking was induced in activated sludge by certain feeding procedures along with *Sphaerotilus* inoculations.

In previous papers of this series (6, 7, 8), the similarity of the sewage purification phenomenon by pure culture zooglear bacteria and by normal activated sludge has been demonstrated. The sewage and glucose metabolism of both pure culture zooglear sludges and of plant activated sludges has also been studied and reported (9, 10). It was decided to study *Sphaerotilus natans* sludges in a similar manner to determine any differences in the metabolism of this organism, and to elucidate, if possible, the factors involved in sludge bulking and the accompanying overgrowth of the zooglear bacteria by *Sphaerotilus*.

PRELIMINARY EXPERIMENTS

A number of early experiments were carried out in cooperation with the biological laboratory upon the growth requirements of *Sphaerotilus natans*. These experiments showed that the fungus had difficulty using glucose in a medium containing only glucose and mineral salts. If nitrogenous materials such as peptone, urea, many amino acids, or sterile domestic or synthetic sewage were added, the rate of growth and glucose utilization was greatly accelerated. In one such experiment in a medium containing glucose and mineral salts, only 41 p. p. m. out of 1,000 p. p. m. of glucose originally present, or 4.1 percent, were used in 120 hours by a *Sphaerotilus* culture. With settled sewage, however, the fungus was able to act upon 800 to 900 p. p. m. of glucose within 24 to 48 hours after inoculation. These experiments also indicated that the glucose attack by *Sphaerotilus* was, curiously, more vigorous when freshly inoculated than when concentrations of 200 to 500 p. p. m. of 48 to 72 hour cultures were used. It was also noted in one experiment that lactic acid was produced. However, lactic acid was not always produced in the metabolism of *Sphaerotilus natans*, and whether its production is due to a change in the metabolism under certain conditions, or to a special strain which cannot be differentiated morphologically from the common strains, is unknown at present.

Lackey and Wattie (2) also isolated a number of other fungi having the general macroscopic appearance and characteristics of *Sphaerotilus*. Experiments with pure cultures of three such strains of fungi indicated that these organisms attack glucose in glucose-sewage media at rates similar to *Sphaerotilus natans*.

FIRST EXPERIMENTS UPON OXYGEN UTILIZATION

A number of experiments to determine oxygen utilization rates were made in 1938 and 1939. The methods employed in the previous work upon zoogleal and plant activated sludges were used. Three bottles containing equal concentrations of *Sphaerotilus natans* were prepared. Two of these were dosed with fresh nutrient material and the third containing the original supernatant was used as a control. The oxygen utilization was then followed in the control and one of the fed culture bottles, while the liquor in these bottles was aerated by mercury pumps at rates of about 1.2 cu. ft. per hour. The *Sphaerotilus natans* solids and glucose content were followed in the third bottle, this bottle being aerated with compressed air. It was found that while this system of study had been satisfactory for the metabolic study of activated sludge and zoogleal bacteria cultures, it was not satisfactory for *Sphaerotilus natans*. The growth and metabolic rates were different in the bottles aerated by compressed air and by the mercury pump, apparently because of differences in some important factor or factors in the two bottles. A condensed summary of the results obtained in six of these experiments is given in table 1. If the results in this table are studied, they will be found to be somewhat inconsistent. Nevertheless, a number of interesting observations may be made from them. First, there seems to be no correlation between the initial quantity of *Sphaerotilus natans* and the quantity of glucose attacked or the extent of *Sphaerotilus* growth during aeration. Second, considering the very high B. O. D. of the feed used in these experiments the quantity of oxygen utilized by the fed culture seems to be low while the quantity used by the control seems rather high. Consequently, the increment of oxygen which was used as a result of the addition of the food appears low. This increment seems to bear no consistent relation to the quantity of glucose acted upon.

TABLE 1.—Glucose removal and oxygen utilization by *Sphaerotilus natans* cultures
(Results obtained by simultaneous aeration in 3 bottles)

Experiment No.	Initial values			After 23 hours of aeration of fed culture		Oxygen utilized in 23 hours of aeration		
	pH	<i>Sphaerotilus natans</i> solids	Glucose	<i>Sphaerotilus</i> solids	Glucose removed	By fed culture	By control culture	As result of food added
		P. p. m.	P. p. m.	P. p. m.	P. p. m.	P. p. m.	P. p. m.	P. p. m.
6.....	6.6	90	503	+148	91	77	19	58
7.....	6.4	418	1,097	+120	527	401	152	249
8.....	6.0	1,304	1,040	+468	831	-----	241	-----
9.....	6.8	1,532	1,083	+334	404	390	283	107
10.....	6.6	1,275	1,053	-229	853	325	256	69
13.....	6.8	153	1,311	+215	173	207	-----	-----

It is interesting to compare the quantities of oxygen used by 1-gram quantities of control zooglear sludge, activated sludge, and *Sphaerotilus natans* sludge as shown below:

Observed oxygen utilization range in mg. O₂ per gram of control sludge in 24 hours

Pure culture zooglear sludge	Plant activated sludge	Pure culture <i>Sphaerotilus natans</i> sludge
16.4 to 29.2.....	37.8 to 177.0....	185 to 364

The differences in the 24-hour oxygen requirements of these three kinds of control sludges is very striking. The very high values for *Sphaerotilus natans* can undoubtedly be explained by two facts. The first is the much higher B. O. D. of the supernatant remaining in such cultures when developed rapidly in sterile sewage glucose media, and the second is the inability to remove as large a fraction of the supernatant aseptically in such cultures owing to the bulkiness and lack of ability of *Sphaerotilus natans* to settle and compact well in the allowable settling period. These experiments indicated that this method of study of *Sphaerotilus natans* metabolism was not satisfactory because of interfering factors which required investigation.

FACTORS AFFECTING THE GROWTH AND METABOLIC ACTIVITIES OF *SPHAEROTILUS NATANS*

Activated sludge plant operation efficiency is affected by such factors as the rate of aeration, pH, temperature and the dissolved oxygen content of the mixed liquor. At present the conditions obtaining in an activated sludge that favor the rapid accumulation of *Sphaerotilus* and the development of a bulky sludge are not well understood. Information as to the conditions which favor the optimum operation of the metabolic processes of *Sphaerotilus natans* and

consequently those which favor the rapid development of *Sphaerotilus* would be of value in determining the conditions under which bulking is not likely to occur. The previous experiments gave evidence that the above factors also affect the development of *Sphaerotilus natans*. Consequently, their effect upon the growth and metabolic processes of pure cultures of *Sphaerotilus natans* was studied in a series of experiments.

EXPERIMENTAL PROCEDURE IN A STUDY OF GROWTH FACTORS

The medium which had been found to contain ample quantities of all the nutrient materials for *Sphaerotilus natans* was used in all of these experiments. This medium contained the following materials:

	<i>Mg.</i>
Dextrose.....	1,000
Peptone.....	600
Meat extract.....	200
Urea.....	50
Na ₂ HPO ₄	50
NaCl.....	15
CaCl ₂	7
MgSO ₄	5
KCl.....	7
Distilled water to make 1 liter.	

Sixteen liter batches of the above medium were prepared and siphoned into each of five 4-liter serum bottles, the pH was adjusted to the desired point, and the bottles of media were sterilized. At the start of each experiment each bottle of medium was inoculated from a thriving 24-hour room temperature culture of *Sphaerotilus* in similar media. The culture used for inoculation contained from 268 to 1,300 p. p. m. of *Sphaerotilus* when determined as dry suspended solids.

While all plantings were made from pure cultures with sterile pipettes and the precautions used on zooglear cultures to maintain pure cultures throughout the 24-hour aeration period were used, bacterial infections sometimes occurred. Usually a 50-ml. portion of the culture was used to inoculate each 4-liter bottle at the beginning of the experiment. Several *Sphaerotilus* strains were used. All of them were very much alike so far as metabolism was concerned except one strain (S-7) which, unlike the others, produced large quantities of lactic acid from glucose.

EFFECT OF AERATION RATE UPON *SPHAEROTILUS NATANS* GROWTH AND METABOLISM

Because it had been noticed in earlier work that the aeration rate affected the growth of *Sphaerotilus natans*, this factor was studied first. Experiments to determine whether *Sphaerotilus natans* was capable of anaerobic growth were included. Three experiments were run at room temperature. Fifteen rates of aeration varying from 0.0 to 11.8 cu. ft.

of air per hour per 3 liters of culture were used. The aeration rates were measured at the start and after 3 to 4 hours and some variations over the 24-hour period were unavoidable. Of the rates used four were less than 0.2 cu. ft. per hour, three in the range of 0.2 to 0.5 cu. ft. per hour, three between 1.0 and 3.0 cu. ft. per hour, and three greater than 5.0 cu. ft. per hour.

At the start and after 24 hours of aeration, examinations were made for bacterial infection, pH, glucose, and *Sphaerotilus* suspended solids. In one experiment total nitrogen determinations were also made. The dissolved oxygen of the aeration mixture was run immediately at the end of each experiment.

The analytical results obtained are given in table 2. These results indicate that most strains of *Sphaerotilus* utilized glucose and peptone with only a small drop in pH (from 6.9–7.1 at the start to 6.4–6.6 after 24 hours). In experiment S-23 with strain S-7, which is the lactic acid-producing strain, the pH dropped from 7.2 to 4.6 and affected the results obtained. For the nonacid-producing strains, as rates of aeration increased from 0.0 to 0.28 cu. ft. per hour, the quantity of *Sphaerotilus* solids produced increased from 17 to 598 p. p. m., above which rate there was no further definite trend. With the *Sphaerotilus* "increase factor," that is, the ratio of *Sphaerotilus* solids at the end to the solids at the start, there was a general rise as the rate of aeration increased to about 3.0 cu. ft. per hour. Above that aeration rate there was no further rise in the "increase factor." The percentage of glucose removed (or attacked) also increased as the rate of aeration increased to about 1.36 cu. ft. per hour, and with greater rates there was a tendency for this percentage to fall slightly. The ratio of the quantity of glucose removed to the *Sphaerotilus* solids produced increased with the rate of aeration up to a rate of 0.48 cu. ft. per hour, the trend for higher rates being erratic. The maximum percentage of total nitrogen taken up was obtained with an aeration rate of 1.36 cu. ft. per hour. The dissolved oxygen in the aerating cultures after 24 hours increased gradually as the rate of aeration increased to 2–3 cu. ft. per hour, above which rate dissolved oxygen values of 6.83 to 7.60 were obtained.

The conclusion is that *Sphaerotilus natans* grows and carries on metabolic processes most efficiently at aeration rates of 0.5 to 3.0 cu. ft. per hour, as evidenced by the highest percentage of glucose removed, the greatest *Sphaerotilus* increase factors, the maximum total nitrogen uptake, and the maximum total nitrogen removed to glucose removed ratio. If the aeration rate of 2.85 cu. ft. per hour of experiment S-21 in which there was considerable bacterial contamination with the probable resultant utilization of oxygen is omitted from consideration, it appears that *Sphaerotilus natans* grew best at aeration rates providing at least 6.0 p. p. m. of dissolved oxygen at the end of the aeration period.

Sphaerotilus can grow and develop appreciably in substrates containing very low quantities, 0.1 to 2.0 p. p. m., of dissolved oxygen. It is significant that in a good medium *Sphaerotilus* can produce up to 598 p. p. m. of solids and utilize up to 600 to 700 p. p. m. of glucose at the low rates of aeration that are required to maintain these low dissolved oxygen values.

SPHAEROTILUS GROWTH IN THE ABSENCE OF OXYGEN AND WHEN NITROGEN IS USED FOR AGITATION

When nitrogen gas from a commercial tank was passed through inoculated media for 24 hours, increases in *Sphaerotilus* were also observed. In two experiments, 61 and 99 p. p. m. of *Sphaerotilus* solids were produced and 32 and 144 p. p. m. of glucose were taken up. The data obtained are also given in table 2. Analysis of the nitrogen gas showed oxygen as an impurity to the extent of 1.04 percent by weight. In another experiment the nitrogen gas was bubbled through a 2-ft. column of alkaline pyrogallol before passing through the *Sphaerotilus* inoculated medium. In this case an 11-p. p. m. increase in *Sphaerotilus* solids was obtained, but no reduction in the glucose was noted in 24 hours. These experiments with nitrogen are interpreted as indicating again the ability of *Sphaerotilus* to grow at very low oxygen tensions.

To determine whether growth was possible in the absence of any oxygen, a liter bottle of media was aseptically deoxygenated with N_2 gas to a dissolved oxygen content of 0.37 p. p. m. and *Sphaerotilus natans* was inoculated into it. The deaerated inoculated medium was siphoned aseptically to fill completely two sterile 1-liter pyrex glass-stoppered bottles. The stoppers were inserted and the sterile tinfoil replaced over them. The bottles were then placed in a turning machine and rotated, end over end, at one revolution per minute, for 24 hours. One bottle was then removed and sampled for dissolved oxygen, glucose, pH, and bacterial determinations. The first bottle was refilled from the second and the rotation was continued for another 48 hours.

After a total of 72 hours the sample was not contaminated and contained only a few small clumps of *Sphaerotilus*. The dissolved oxygen was 0.0, the pH 7.0, and the glucose content 1,056 p. p. m., indicating no change in this constituent. Glucose removal did not occur during either of the periods. The *Sphaerotilus* solids increased from 23 to 40 p. p. m. during the first 24 hours, indicating a very slight growth, but no further growth occurred in the remaining 48 hours. These results indicate that *Sphaerotilus natans* is capable of growing to a slight extent in a good medium at extremely low oxygen tensions but is unable to grow in the absence of oxygen.

EFFECT OF pH UPON *SPHAEROTILUS* GROWTH

In these experiments small quantities of 10 percent H_3PO_4 , or 10 percent NaOH, were added to bring the pH to the desired point. The 4-liter bottles containing the media were sterilized, and before inoculation the pH was again checked and adjusted if necessary. All bottles were aerated for 24 hours at a rate as near 1.0 cu. ft. per hour as it was possible to maintain. Three experiments were run, one with a pH range from 3.0 to 7.0, and two with a range from 7.0 to approximately 10.0.

The analytical results obtained are given in table 3. These data indicate that aeration at a pH below 5 was detrimental to *Sphaerotilus* growth and metabolism. Glucose and nitrogen uptake was apparently completely stopped. Only 35 to 42 p. p. m. of *Sphaerotilus* were produced in 24 hours at these pH values. The high dissolved oxygen (7.75 to 7.90 p. p. m.) at the end of the experiment indicates that little oxygen was used.

Even at a pH of 6.0 the activity of *Sphaerotilus* was partially stopped. This is shown by the solids produced, glucose removed, and nitrogen uptake data. At this pH, 253 p. p. m. of *Sphaerotilus* were produced, 495 p. p. m. of glucose were removed, and 22 p. p. m. total nitrogen taken up.

The most favorable pH is the range from 6.6 to 9.0, as evidenced by a *Sphaerotilus* solids production of 480 to 635 p. p. m., a glucose utilization of 826 to 1,040 p. p. m. (71.3 to 90.3 percent), a total nitrogen uptake of 27.9 to 34.2 p. p. m., and by the highest ratios of glucose used to nitrogen used and solids produced to nitrogen used.

TABLE 3.—Effect of pH of medium on the metabolism of *Sphaerotilus natans*

Rate of aeration, cubic feet per hour.....	1.03	0.99	1.10	1.18	1.0	1.0	1.0	1.0	
								First 24 hours	Next 24 hours
pH (Initial.....)	3.90	4.95	6.05	7.05	8.0	9.0	+9.6	+9.6	8.4
(After 24 hours.....)	3.85	5.85	5.85	6.45	7.0	7.4	7.4	8.4	6.9
<i>Sphaerotilus</i> suspended solids:									
Initial.....	16.0	16.0	16.0	32.0	2.2	2.2	2.2	13.0	-----
Amount produced in 24 hours.....	37.0	35.0	253.0	480.0	497.0	585.0	510.0	39.0	-----
Glucose attacked, p. p. m., initial concentration between 1,100-1,200 p. p. m.	3.0	36.0	495.0	826.0	992.0	993.0	996.0	14.0	578.0
Percentage nitrogen taken up.....	0	2.8	15.7	24.2	25.3	22.7	24.5	0	-----
Mg. glucose attacked per mg. <i>Sphaerotilus</i> produced.....	.081	1.02	1.96	1.72	2.0	1.69	1.75	.36	-----
Mg. glucose used per mg. nitrogen taken up.....	-----	9.0	22.5	34.1	29.0	32.3	30.1	-----	-----
Mg. <i>Sphaerotilus</i> solids produced per mg. nitrogen taken up.....	-----	8.75	11.5	14.1	14.5	19.0	15.4	-----	-----
Dissolved oxygen content after 24 hours.....	7.90	7.75	4.60	.74	3.50	3.21	2.27	-----	-----

In one experiment, good growth occurred in a medium with an initial pH of about 9.6-10.0 and it was observed that the pH had dropped to 7.4 after 24 hours. In another experiment (last two columns in table 3) with the same initial pH there was only slight growth in the first 24 hours during which the pH had fallen to only 8.4. In the next 24 hours, however, considerable growth occurred, 578 p. p. m. of glucose were utilized, and the pH fell to 6.9. This seems to indicate that pH values between 8.5 and 10.0 have an inhibitory effect, but that the organisms are able to produce acid in sufficient quantities to bring the pH to a more favorable range for further growth.

EFFECT OF AERATION TEMPERATURE UPON GROWTH OF *SPHAEROTILUS*

The bottles of medium were prepared as before and each bottle was stored overnight at the temperature at which it was to be aerated. The tests at 10°, 15°, 20°, and 37° C. were carried out in incubators at these temperatures. The bottle which was to be aerated at 30° C. was aerated in an alberene stone hood which was maintained at 30° C. by radiation from a muffle furnace. The temperature in the hood varied slightly but remained between 29° and 31° C. most of the time. The aeration rates in this study were approximately 1.0 to 1.2 cu. ft. per hour. The experiments were carried out in the same manner as when aeration rates and pH were being studied.

TABLE 4.—Effect of incubation temperature on the metabolism of *Sphaerotilus*

Incubation temperature °C.....	10	15	20	30	37	
Rate of aeration, cu. ft. per hour.....	1.24	1.0	1.0	1.23	1.0	
pH { Initial.....	7.0	7.0	7.0	7.0	7.0	
{ Final (24 hours).....	7.0	6.7	6.0	6.8	5.4	
<i>Sphaerotilus</i> suspended solids, p. p. m. {	Initial.....	8.5	1.8	1.8	8.5	1.8
	Amount produced in 24 hours.....	8.0	133.0	989.0	708.0	390.0
Glucose removed, p. p. m. in 24 hours.....	17.0	44.0	633.0	1,003.0	533.0	
Total nitrogen removed, p. p. m. in 24 hours.....	0	6.7	20.9	42.9	19.1	
Mg. glucose used per mg. solids produced.....	2.12	0.33	0.64	1.41	1.42	
Mg. glucose used per mg. nitrogen used.....	6.55	30.3	23.4	28.9	
Dissolved oxygen after 24 hours.....	5.96	2.68	3.92	

The results of the temperature experiments are given in table 4. At 10° C. only very slight growth occurs, as shown by the fact that only 8 p. p. m. of *Sphaerotilus natans* were produced and only 17 p. p. m., or 1.5 percent, of glucose were removed in 24 hours. At 15° C. the ability to grow is somewhat better but even at this temperature only 133 p. p. m. of *Sphaerotilus* solids were produced and 44 p. p. m. of glucose were removed. In the two tests at 20° C., 533 and 633 p. p. m. of glucose were removed and 370 and 989 p. p. m. of *Sphaerotilus* were produced. A drop to pH 5.4 in the first test probably accounts for the low *Sphaerotilus* yield. The yield of 989 p. p. m. of solids in the second test is higher than the average yield of about 490 p. p. m. for the 14 tests that were run at optimum aeration rates and pH values at 20° C. and at room temperature.

A temperature of 30° C. was most favorable for the growth of *Sphaerotilus natans*. At this temperature about 1,000 p. p. m., or over 86 percent, of the glucose was removed and about 700 p. p. m. of *Sphaerotilus* solids were produced. A fall in the glucose uptake to 437 and 553 p. p. m. and in solids production to 280 and 390 at 37° C. indicates that this temperature is above the optimum for this organism.

SPHAEROTILUS NATANS METABOLISM

In another series of experiments very small quantities of *Sphaerotilus natans* were inoculated into the sterile peptone glucose medium and two bottles were aerated simultaneously at 20° C., one by compressed air and the other by the mercury pump. In the last two of these experiments a small piston pump which was specifically designed for aeration of small quantities of liquids was used in place of compressed air. In the bottle aerated by the mercury pump, pH, glucose content, *Sphaerotilus natans* content, and B. O. D. of the supernatant were determined at the beginning and the end of the experiment, and the quantity of oxygen used was determined at regular intervals. In the bottle in which compressed air or the small piston pump was used for aeration the same determinations were made at intervals but

in place of oxygen used the carbon dioxide produced was determined. A summary of the analytical data obtained is given in table 5.

TABLE 5.—Summary of analytical data on growth and metabolism of *Sphaerotilus natans*

Seven experiments, 24-58 hour aeration periods	<i>Sphaerotilus</i> solids produced, p. p. m.	Glucose removed, p. p. m.	Oxygen used, p. p. m.	Carbon dioxide produced, p. p. m.	L value removed, p. p. m.
Compressed air aeration					
Maximum values.....	799	931	1 616	848	1936
Minimum values.....	152	197	77	106	136
Mean values.....	387	464	220	303	795
Mercury pump aeration					
Maximum values.....	544	986	550	1 756	1 959
Minimum values.....	206	292	105	144	146
Mean values.....	332	522	241	332	569

¹ Oxygen equivalent of CO₂ used.
² CO₂ equivalent of oxygen used.

³ Data of five experiments only.
⁴ Data of three experiments only.

In experiment 15 the aeration periods were different and checks in the analytical data cannot be expected. The mercury pump aerated cultures usually produced the least *Sphaerotilus natans*. In all of these experiments an average of 322 p. p. m. of *Sphaerotilus* were produced by them, compared to 387 p. p. m. for the compressed air unit. The mercury pump aerated cultures, however, removed an average of 522 p. p. m. of glucose compared to 464 for the compressed air aeration. By calculating oxygen equivalents of the CO₂ produced in the compressed air system the oxygen utilization in the two systems may be compared. Large differences in the quantities of oxygen utilized and CO₂ produced in experiments with the same aeration periods were obtained only in experiments 17 and 20.

The metabolic changes produced per unit of *Sphaerotilus* formed are given in table 6. While the quantity of glucose removed per mg. of *Sphaerotilus* produced varied considerably in the individual experiments, each result obtained is within the range of these values given in tables 2, 3, and 4. In the present experiments from 0.535 to 3.546 mg. of glucose were required per mg. of *Sphaerotilus* produced, while in table 2 this ratio varied from 0.37 to 3.81. The mean quantity of glucose used per mg. of *Sphaerotilus* formed for the mercury pump was 1.752 mg. compared with the mean of 1.242 for the compressed air experiments. Slightly higher L values removed per unit of *Sphaerotilus* formed (1.969 and 1.636 for the mercury pump and compressed air systems, respectively) were obtained, as would be expected.

In these experiments, with periods of aeration of 24 hours and longer, an average of about 0.67 mg. of CO₂ was produced for each

mg. of glucose removed. The quantity of CO₂ produced per mg. of *Sphaerotilus* produced varied from 0.296 to 2.183 mg. with a mean of about 1.0 mg.

TABLE 6.—Summary of transformations produced by *Sphaerotilus natans* cultures

Seven experiments—24-58-hour aeration periods	Mg. glucose used per mg. <i>Sphaerotilus</i> solids produced	Mg. CO ₂ produced per mg. glucose used	Mg. CO ₂ produced per mg. solids produced	Mg. B.O.D. (L value) removed per mg. solids produced	Mg. solids produced plus glucose equivalent of CO ₂ produced per mg. glucose used
Compressed air aeration					
Maximum values.....	1.98	¹ 1.06	¹ 1.81	² 2.42	² 2.04
Minimum values.....	.585	.373	.296	.575	.811
Mean values.....	1.24	.681	.930	1.64	1.34
Mercury pump aeration					
Maximum values.....	3.55	⁴ 1.264	⁴ 2.18	² 2.77	⁴ 2.24
Minimum values.....	.535	.917	.296	.709	.416
Mean values.....	1.75	.668	1.072	1.97	1.25

¹ Data of six experiments only.
² Data of three experiments only.
³ Data of five experiments only.
⁴ On basis of CO₂ equivalent of oxygen used.

It was learned also from experiments 14, 15, and 16 that more glucose was used per unit of *Sphaerotilus* produced as the aeration

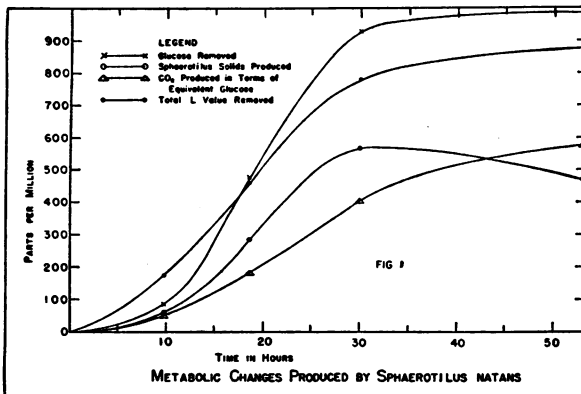


FIGURE 1

period was increased. With *Sphaerotilus natans* the metabolic activity apparently changes somewhat with the age of the culture, resulting in less synthesis and more respiration. No similar evidence of such a change in metabolism could be detected in our earlier studies with zoogeal sludge or plant activated sludge. The growth of *Sphaerotilus natans* and the chemical changes produced in the medium have been plotted in figure 1 for experiment 16 which is considered typical.

NUTRITIONAL EXPERIMENTS WITH PLANT ACTIVATED SLUDGE TO
INDUCE BULKING

A series of experiments was carried out with plant activated sludge to study the joint effect of *Sphaerotilus natans* and variations in nutrition in inducing bulking. As these experiments progressed, changes in the procedures were made twice so that the experiments are divided into three parts.

EXPERIMENTAL PROCEDURE

Plant sludge was distributed in 8-liter quantities into six 10-liter serum bottles labeled A to F. Bottles A, B, C, and D received quantities of pure culture *Sphaerotilus natans* and cotton-filtered domestic sewage every day. In addition, B, C, and D received 200, 500, and 1,000 p. p. m. of glucose daily, whereas bottle A received no glucose. Bottle E received *Sphaerotilus natans* and 500 p. p. m. of glucose in distilled water but no nitrogenous or mineral material. Bottle F received filtered sewage plus 500 p. p. m. of glucose but no *Sphaerotilus natans*. The above feeding procedure may be outlined as follows:

Bottle containing plant sludge labeled	Filtered sewage	Glucose dose, p.p.m.	<i>Sphaerotilus natans</i>
A-----	+	0	+
B-----	+	200	+
C-----	+	500	+
D-----	+	1,000	+
E-----	-	500	+
F-----	+	500	-

The analytical work included settling tests, pH, suspended solids, and ash determinations. Twice each day, in the morning before feeding and in the afternoon 4 to 5 hours after feeding, sludge volumes at 10-minute intervals for a period of 1 hour were determined. Sludge temperature and pH were recorded at the time the settling tests were made. Suspended solids and ash were determined every morning before feeding, and the sludge index, according to Mohlman (11) was calculated. A *sludge looseness index* was also calculated. This we define as the sludge volume of a liter sample after a 10-minute settling period divided by the volume of the supernatant after a 60-minute settling period, or as
$$\frac{10' \text{ sludge volume}}{1,000-60' \text{ sludge volume}}$$

Each morning before feeding the air was turned off and each sludge allowed to settle for 1 hour after the 1-liter samples for the settleable solids test had been removed. As much as possible of the supernatant was siphoned off and the feeding procedure and *Sphaerotilus* inoculation carried out.

Each day a 24- to 48-hour room temperature culture of *Sphaerotilus* in a glucose peptone medium was used for inoculating the sludge. The 8-liter culture was settled and the concentrated solids were apportioned equally into each of the five bottles being inoculated.

After inoculation and feeding each bottle was aerated, at room temperature, at a rate of 3½ to 4 cu. ft. per hour per 6 liters of sludge (the minimum rate that would keep these sludges entirely in suspension). The glucose content of all sludge mixtures was determined 3 hours after feeding and again in the morning just before the settling, inoculation, and feeding procedures were repeated.

The total quantities of *Sphaerotilus natans* solids added to each bottle, by respective dates, were:

Total Sphaerotilus added, by respective dates

(Grams, dry solids)

Bottle	June 29	June 30	July 1	July 2	July 3	July 4	July 5	July 6	July 7
A	0.594	1.209	3.381	3.687	4.309	4.655	5.192	5.472	5.788
B	.594	1.209	3.381	3.687	4.309	4.655	5.192	5.472	5.788
C	.594	1.209	3.381	2.097	2.719	3.065	3.602	3.882	-----
D	.594	1.209	2.777	2.011	2.357	2.894	-----	-----	-----
E	.594	1.209	3.381	2.097	2.719	3.065	3.602	-----	-----
F	(¹)	(¹)	(¹)	(¹)	(¹)	(¹)	(¹)	(¹)	(¹)

¹ None.

As indicated, the values for total *Sphaerotilus* (cumulative) added on the respective dates vary somewhat even though similar inoculations were given to all bottles on each day. Because C, D, and E bulked, some sludge had to be wasted and the proportion of *Sphaerotilus* lost in wasting sludge was calculated. The figures do not indicate the quantities of *Sphaerotilus natans* present on these dates. The actual quantities of *Sphaerotilus* in the sludge could not be determined. However, counts of the *Sphaerotilus* flocs found per ml. of these sludges were made which indicated definite trends. These trends may be summarized as follows: After inoculation the first day from 1,152 to 2,816 flocs per ml. were found in these sludges. In sludge A, which received *Sphaerotilus natans* but no sugar, the count dropped after every feeding. In samples B and C, which received 200 and 500 p. p. m. of sugar respectively, no evidence of growth of the *Sphaerotilus natans* was found. In sludges D and E there was evidence that the *Sphaerotilus* remained viable but at the end of the experiment the numbers of flocs were no greater than at the start. In sludge F, which received 500 p. p. m. of glucose with sewage but no *Sphaerotilus*, very small numbers of *Sphaerotilus* flocs were found (64 to 128), and there was no tendency for the *Sphaerotilus* count to increase. The *Sphaerotilus natans* observed in F was present in the original sludge taken from the plant.

FACTORS INVOLVED IN BULKING

The effect of glucose and *Sphaerotilus natans* in inducing bulking is shown in figure 2. The graph records the change in sludge indices over the 9-day period of this experiment. As indicated by the very low indices for sludge A, *Sphaerotilus* alone will not induce bulking.

Glucose (when fed jointly with sewage) without *Sphaerotilus* induced slight bulking after the second day as indicated by sludge F.

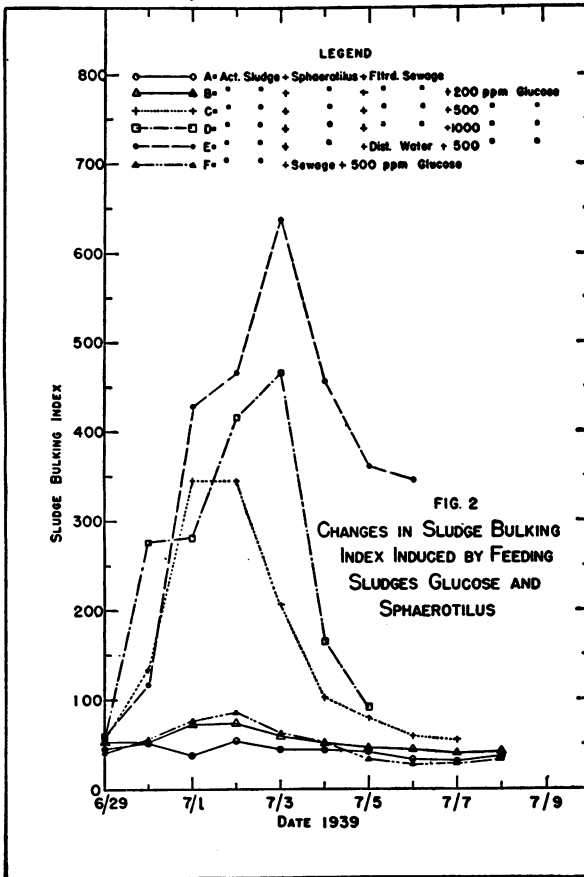


FIGURE 2

After 5 days of feeding with 500 p. p. m. of glucose in sewage, this sludge had completely recovered. *Sphaerotilus natans* and 200 p. p. m. of glucose also induced very slight bulking in B, but recovery occurred after the third day. However, *Sphaerotilus* and a glucose dose of 500 p. p. m. with sewage induced immediate bulking in C from which recovery, while slower, did occur after the seventh day. With a glucose dose of 1,000 p. p. m. (D) a maximum index of 465 was obtained after the fourth day which then rapidly dropped to less than

100 at the sixth day when the sludge was used for total purification and oxidation tests.

Glucose in distilled water plus *Sphaerotilus* was the most vigorous vector in inducing bulking. The sludge index of E fed in this manner increased from 50 to 270 the first day and reached a maximum of 640 in 5 days, after which it dropped gradually, but was still above 300 on the seventh day when this procedure was discontinued.

In figure 3 the looseness indices for this experiment have been plotted on a log scale with time. This shows the changes involved as effec-

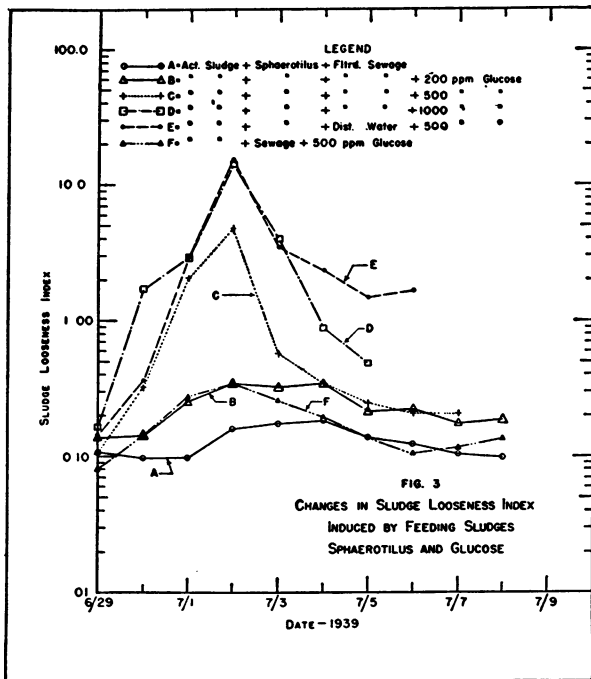


FIGURE 3

tively as the previous graph. This looseness index seems to be a more sensitive indicator of difference for cases of low sludge index.

The changes in the percentage of ash in the sludge have been plotted in figure 4. The low ash content is the result of feeding low ash *Sphaerotilus natans* and glucose.

Unfortunately our data are incomplete in respect to changes in glucose removal rates during the period when bulking was most rapid. However, in all sludges but E there was a gradual increase in the percentage removed in 3 hours. By the fifth day, A, B, C, and F (A received some glucose with the *Sphaerotilus* inoculum) were able to remove 90 to 100 percent of all glucose added in 3 hours. On the other hand, E, which received no organic nitrogen or minerals for metabolism, deteriorated in its glucose-removing ability, the per-

centage acted on in 3 hours falling from 30.4 percent after the first day to 4.3 percent on the sixth day. Sludge D, which received sufficient mineral and nitrogenous material with the glucose, removed 51.9 percent in 3 hours even when it was bulking severely.

After 9 days of treatment as described above, sludges A, B, and F were each divided between two 10-liter aeration bottles and each pair was treated as previously. One series of bottles designated A₁, B₁, and F₁, were aerated at the previous rate while the second series designated A₂, B₂, and F₂, were aerated at 10 to 11 cu. ft. per hour. All the previous tests were run and this experiment was continued for 4 days, at which time a total of 3.449 grams of *Sphaerotilus* solids had been added to each of the A and B series bottles. The F bottles received 500 p. p. m. of glucose with sewage daily but no *Sphaerotilus*.

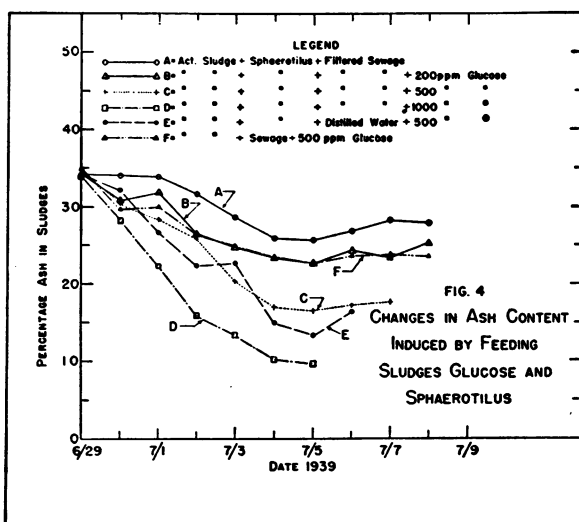


FIGURE 4

No additional bulking was noted in any of these sludges during this experiment. Increasing the rate of aeration to 10 cu. ft. per hour in the sub 2 series bottles showed no effect upon the sludge indices, but did increase somewhat the looseness index of these sludges.

THE EFFECT OF NUTRITIONAL TREATMENT AND BULKING UPON THE BIOCHEMICAL CHARACTERISTICS OF THE SLUDGE

After various periods of observation, the sludges receiving *Sphaerotilus* and varied nutritional treatment as described were taken for determination of their biochemical qualities. The over-all purification rate, the oxidation capacity, and sludge demand of each sludge were measured. All of these determinations were made as previously described (8). The total purification was determined with sewage

alone and also with sewage fortified with 500 p. p. m. of glucose. The oxidizing capacity was determined upon sewage alone. These tests were made upon sludge D after it had been under observation for 6 days, and similar tests were made upon the other sludges in the following order: E, C, F₂, B₂, and A₂. The data obtained on total or over-all purification are given in table 7, and the percentages of L value of domestic sewage removed are plotted in figure 5. The

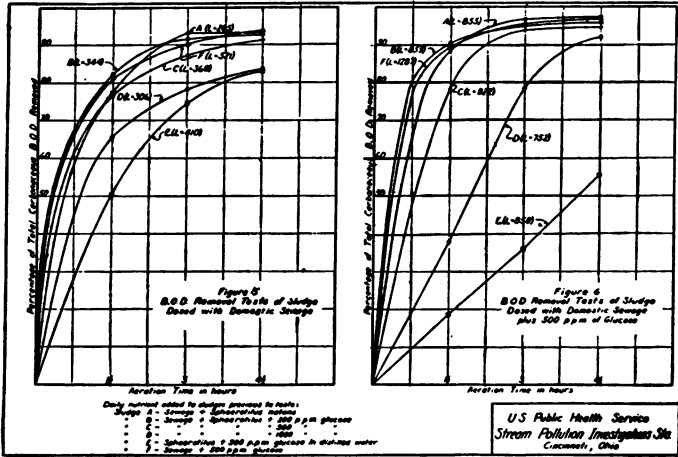


FIGURE 5

FIGURE 6

previous treatment and bulking had a decided effect in lowering the over-all purification for the first 1½ to 3 hours in the cases of sludges C, D, and E, the detrimental effect being greatest for sludges D and E. The L value removal of sewage plus glucose was still more deteriorated in these sludges for the first 1½ hours, as shown in figure 6. This deterioration is again greatest with E. The good sludges A₂, B₂, and F₂, on the other hand, were able to remove the B. O. D. of sewage plus glucose at a slightly greater rate than that of sewage alone.

TABLE 7.—Differences in biochemical characteristics of sludges induced by *Sphaerotilus* and variations in feeding procedure

Sludge	Days fed before testing	Sludge index	Suspended solids present, P. P. M.	Volume of sewage feed per liter of mixture, in ml.	Nature of test feed	Transformation of L value						Removal of glucose				Sludge demand, P. P. M. per gram sludge in indicated time (hours)				
						Percentage removed (hours)			Percentage oxidized (hours)			Glucose present initially, P. P. M.	Percentage removed (hours)			1½	3	4½		
						1½	3	4½	21	1½	3		4½	1½	3				4½	
D	5	91.8	2,776	650	(S ¹ + G ¹)	65.7	78.3	83.8	90.1	11.6	19.0	24.2	680	33.8	91	100	13.4	23.7	30.5	
E	6	347	1,800	760	(S + G)	37.8	78.3	91.9	94.3	4.17	11.2	18.3	647	1.64	20.0	97.5	15.8	22.0	28.6	
C	7	5.1	3,088	780	(S + G)	50.5	74.6	83.1	89.2	8.28	11.7	15.5	---	---	---	---	10.8	15.9	16.5	
F ₁	10	24.8	2,620	800	(S + G)	76.6	87.3	91.1	93.7	8.57	10.6	16.4	---	---	---	---	3.47	6.98	11.0	
B ₁	11	35.8	3,638	800	(S + G)	81.2	90.3	94.0	99.3	7.29	9.94	24.7	779	90.4	96.4	100	9.52	12.4	17.1	
A ₂	12	36.4	2,608	800	(S + G)	82.4	95.5	96.7	99.3	17.8	25.8	33.5	535	80.6	92.6	100	8.63	15.8	19.3	
					(S + G)	77.6	93.3	93.4	97.3	---	---	---	416	96.8	100	---	---	---	---	---
					(S + G)	88.3	96.7	97.2	98.8	---	---	---	---	---	---	---	---	---	---	---

¹ Sewage alone.

² Sewage plus 500 P. P. M. glucose.

The decrease in the over-all purification performance of sludges D and E is also correlated with a decrease in the ability to attack glucose. Sludges A₂, B₂, and F₂ were able to remove 80 to 96 percent of the glucose fed them in the sewage plus glucose feed in 1½ hours, and 96 to 100 percent in 3 hours. Sludge D was able to take up only 39 percent in 1½ hours while E removed only 1.6 percent in 1½ hours and 39.6 percent after 5 hours. As glucose is primarily removed through adsorption and synthesis rather than oxidation (9, 10), this would indicate that the deterioration in over-all purification was not correlated with a reduction in the oxidation performance but rather with a deterioration in the adsorption and synthesis mechanism. The actual values for percentage of L value oxidized as given in table 7 seem to corroborate this. The data indicate no correlation between the approximately normal oxidation capacities of sludges D and E and their over-all B. O. D. purification deterioration.

There are no striking differences in the oxidation capacities of any of the sludges that received *Sphaerotilus natans* and glucose. However, sludge A₂, which received *Sphaerotilus natans* but no glucose, seems to be in a class by itself. Upon the basis of percentage of L value oxidized per gram, sludge A gave values of 6.8, 9.9, and 12.8 percent in 1½, 3, and 4½ hours, respectively. This performance is about double that of the mean for all sludges given regular doses of glucose.

There was a considerable variation in the sludge oxygen demand depending upon the previous treatment. The 4½-hour demand per gram of sludge varied from 11 to 19 mg. for the good sludges A₂, B₂, and F₂. Sludge F₂ which received 500 p. p. m. of glucose daily but no *Sphaerotilus natans* had the lowest demand. This would be expected from the pure culture experiments described earlier. Sludge D, which received *Sphaerotilus* and the largest glucose dose daily, had a (4½-hour) demand of 30.6 mg. per gram, the highest of these sludges. Sludge E, which received the same quantity of glucose as F but in distilled water and also received *Sphaerotilus*, had a demand under the same conditions of 28.6, or the second highest.

CONDITIONING OF SLUDGES TO *SPHAEROTILUS* AND GLUCOSE

Sludges A₂ and F₂ from the former experiment were each divided into two portions, A₃, A₄, and F₃, F₄. In addition two bottles of plant sludge, which was bulking somewhat (index 113) but contained little *Sphaerotilus*, were obtained and labeled P₃ and P₄. The sludges A₃, F₃, and P₃ were fed domestic sewage, and sludges A₄, F₄, and P₄ were fed sewage fortified with 1,000 p. p. m. of glucose. Pure culture *Sphaerotilus natans* solids, varying between 0.065 and 0.282 gram, were added to each of the six sludges daily. The experimental procedures employed were identical with those used in the previous series of sludges. This series was carried out for 10 days, at which

time the following amounts of *Sphaerotilus natans* were present in 8 liters of sludge aeration mixture, assuming no loss or gain:

A ₃	3. 229
A ₄	1. 816
B ₃	3. 229
B ₄	1. 816
P ₃	1. 654
P ₄ 989

The sludge indices obtained during this experiment are plotted in figure 7, and the looseness indices are shown in figure 8. With the aeration procedure the same as before, apparently sludge A had become acclimated to *Sphaerotilus* doses and sludge F had become acclimated to glucose. In any case no disturbance in physical characteristics was produced in any of these sludges in this experiment.

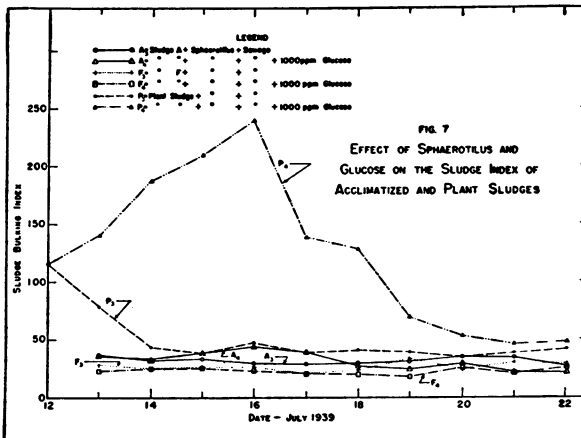


FIGURE 7

Sludges A₃, A₄, F₃, and F₄ all had indices under 50 throughout the experiment and the solids content varied from 2,300 p. p. m. at the start to 6,000 toward the end. In this experiment, therefore, bulking was not induced by *Sphaerotilus* and 1,000 p. p. m. of glucose in sludge A (which had received very little glucose previously), or in sludge F which had received no *Sphaerotilus* previously.

The plant sludge, however, behaved differently. The sludge as received had an index of about 113, indicating mild bulking. The addition of *Sphaerotilus* and sewage to P₃ did not further extend bulking but instead the sludge was able to recover and attained an index of 40 or less within 2 days. However, the addition of *Sphaerotilus* and glucose with sewage immediately intensified bulking, an index of 240 being reached after 4 days, which was followed by a gradual recovery.

Though no change was noted in the sludge index, differences in the looseness indices of A₃ and A₄, and F₃ and F₄ occurred in this experi-

ment. The looseness index of A_4 increased for 3 days and of F_4 for 6 days. Apparently the looseness index is more sensitive than the common index for slight disturbances in the physical structure of the sludge.

These experiments indicated that neither *Sphaerotilus natans* alone nor glucose alone will induce bulking in a well-aerated activated sludge. There may be a slight increase in the sludge index or the looseness index but recovery will be rapid even with continued feeding of glucose or *Sphaerotilus* with sewage. However, when both *Sphaerotilus* and glucose are added together to a sludge which has not previously received them, a disturbance of the sludge occurs and bulking is quickly produced, the extent depending upon the amount of glucose added.

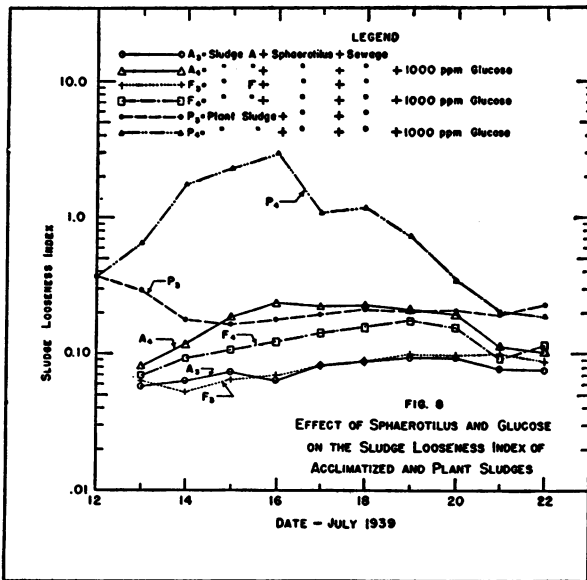


FIGURE 8

Gradually over a 5- to 10-day period recovery will take place even with continued application of the *Sphaerotilus* and glucose. It has been shown that addition of *Sphaerotilus* to a mildly bulking sludge, well aerated, did not intensify bulking, but was followed by recovery. Also, that a *Sphaerotilus* or glucose acclimated sludge well aerated was able to take regular applications of both *Sphaerotilus* and glucose without bulking sufficiently to be detected by changes in sludge index although a slight increase in the looseness index did occur.

DISCUSSION

It has been shown that *Sphaerotilus natans* grows well in an aerated glucose-peptone medium. Under such favorable conditions from 400 to 700 p. p. m. of fungus solids can be obtained in 24 hours from an

inoculum of only a few p. p. m. The medium containing the necessary mineral constituents along with 1,000 p. p. m. of glucose and 600 p. p. m. of peptone had an L value of about double that of a strong domestic sewage. This medium, though designed for the cultivation of *Sphaerotilus*, was also a very favorable substrate for the growth of zooglear bacteria, inocula of 10 to 100 p. p. m. producing 440 to 570 p. p. m. of additional solids after 24 hours of aeration (12). Whereas there were decided variations in the response of different *Sphaerotilus* cultures to growth in this medium, the response of zooglear cultures was more consistent. In any case, no evidence was obtained to indicate that the medium was more favorable to *Sphaerotilus* growth than to zooglear growth.

However, several differences were noted in the metabolic activity and physiology of *Sphaerotilus* and *Zooglea ramigera*. Apparently some strains of *Sphaerotilus* are capable of producing organic end products of metabolism, appreciable amounts of lactic acid being produced even with rates of aeration providing oxygen for all metabolic needs. Most strains are not acid producers. However, in several instances a more rapid removal of glucose than of L value B. O. D. was observed which would indicate the break-up of at least a portion of the glucose into simpler products. Zooglear bacteria, in pure culture or in activated sludge, on the other hand, have never been observed to produce any end products from glucose, when well aerated, other than CO₂ and cellular material.

On the basis of mg. of glucose acted on per mg. of solids produced in pure culture, there is very little difference in the performance of these organisms. These values ranged from 1.74 to 2.94 for zooglear bacteria (12) and from 1.17 to 3.81 for *Sphaerotilus* under the same conditions. The variation is greater in this datum for *Sphaerotilus* than for the zooglear organisms and this greater variation for *Sphaerotilus* was noted in all measurements that were made.

The values for mg. of solids produced per mg. of organic nitrogen taken up were quite different for the two organisms, being 22.5 to 34.1 for *Sphaerotilus natans* and only 5.40 to 6.99 for zooglear bacteria. From this the *Sphaerotilus* might be expected to outgrow these bacteria in a medium containing a very limited amount of organic nitrogen. Another important difference is the much greater short time oxygen demand of *Sphaerotilus natans* control sludges compared to control activated sludges or pure culture zooglear sludges. Finally, the metabolism of *Sphaerotilus* apparently changes somewhat with the age of the culture, but similar changes have not been observed with cultures of zooglear bacteria.

The temperature experiments showed *Sphaerotilus* to have a somewhat narrower optimum growth range than zooglear bacteria or

activated sludge. While the maximum rate of growth occurred at about 30° C., the rate fell off remarkably below 15° C. and was very limited at 10° C. This is undoubtedly the reason for less frequent difficulties with *Sphaerotilus* during the winter months. The optimum pH range for *Sphaerotilus* also was found to differ somewhat from normal activated sludge. *Sphaerotilus natans* grew very well over a pH range from 6 to about 9 and was able to remain viable and slowly reduce the pH at values up to about 10. However, it was very sensitive to pH values below 6.0 and growth was practically inhibited at values below 5.0. It appears much more sensitive to pH values below 6.0 than normal activated sludge.

The oxygen requirements of *Sphaerotilus* is a controversial question. If it were true, as some have claimed, that *Sphaerotilus* is a facultative anaerobe, it would be logical to assume that this organism might have a growth advantage over the strictly aerobic zooglyphic organisms in activated sludges of zero or very low oxygen content. However, our results indicate very decidedly that *Sphaerotilus natans* can grow only under aerobic conditions and is dependent on oxygen as a hydrogen acceptor. In the total absence of oxygen no growth occurs and no glucose is acted upon. At low oxygen tensions growth of *Sphaerotilus* and removal of glucose does take place, but at a very low rate. With increasing rates of aeration the quantity of the organism produced increases rapidly, rates of 3 to 5 cu. ft. per hour per 3 liters of medium being optimum. In this respect the behavior of *Sphaerotilus* is identical with that of the zooglyphic organisms. No evidence could be found that indicated that low rates of aeration were more favorable to the growth of *Sphaerotilus* than higher rates. There is some evidence, however, to indicate that aeration rates in the neighborhood of 10 cu. ft. per hour per 3 liters of medium did hinder *Sphaerotilus* growth somewhat. Such rates are sufficiently violent to break up the *Sphaerotilus* flocs and are much higher than any ever met in practice. It may be possible that at extremely low oxygen tensions *Sphaerotilus* may have some advantage over the zooglyphic organisms, but evidence to indicate or disprove this has still to be obtained.

Zooglyphic bacteria are quite sturdy organisms in that fairly large variations in environmental conditions such as oxygen tension, pH, temperature, and relative ratio of sludge to feed do not appreciably affect their metabolism and their floc properties. However, with extreme and sudden changes there can be expected unfavorable reactions on the part of these organisms toward the change in conditions. *Sphaerotilus*, on the other hand, is a much more delicate organism, small changes in conditions affecting its metabolism appreciably. This is evidenced by the variations in solids produced, glucose uptake, and other data obtained when *Sphaerotilus* was grown under conditions as nearly identical as possible. In other words, *Sphaero-*

tilus will appear in plants in practice only under the most favorable conditions for these organisms. That these are seldom obtained is evidenced by the generally infrequent and temporary appearance of the organism in large quantities.

Because carbohydrates in sewage are a superior food material for the organism, large quantities of *Sphaerotilus* have always been associated with the sudden appearance of abnormal quantities of carbohydrates in plant influents. Under such conditions bulking has frequently been observed. However, bulking cannot be considered as the direct effect of the *Sphaerotilus*, although the interweaving of the sludge flocs with the buoyant *Sphaerotilus* filaments can be a secondary factor in intensifying the bulking effect.

The sudden appearance of carbohydrates acts, in a manner not yet understood, as a shock to the organisms of the activated sludge system. The response to this shock is a biophysical change in floc properties resulting in a light, noncompact, poorly settling floc. It must be understood that bulking and the appearance of *Sphaerotilus* are both responses to the same change in conditions which upset the biological equilibrium, in this case the sudden appearance of carbohydrates. Our latter experiments support this conclusion.

The addition of sizable quantities of *Sphaerotilus* to good, active sludges will not induce bulking. As a matter of fact the *Sphaerotilus* will generally die out rapidly. Feeding glucose in addition to sewage may or may not induce bulking in a sludge free of *Sphaerotilus*. Further, sludges containing *Sphaerotilus* and being fed relatively small quantities of glucose with sewage will bulk slightly, but will immediately recover. Intense bulking will be obtained only when sludges containing *Sphaerotilus* are either fed sewage with very large quantities of glucose or are fed glucose in distilled water. In this latter case, the feeding of the glucose to the sludge without supplementary nitrogenous materials is such a strong "shocking" agent that the bulking condition produced is one from which recovery is very slow.

After sludges have been receiving such abnormal food for some time they become acclimatized and a new biological equilibrium is established for this condition. In our case following acclimatization to glucose the sludge was able to receive and react to 1,000 p. p. m. of glucose even in the presence of *Sphaerotilus* without bulking in any manner. That *Sphaerotilus* is not the direct causative agent of bulking is indicated by the fact that a bulking sludge normally receiving sewage can be fed *Sphaerotilus* and sewage without glucose and the bulking will gradually disappear.

The induction of bulking in sludges apparently does not affect the oxidizing capacity of the sludge. In other words, bulking is not associated with any change in the physiology of the zooglyphic bacteria. However, bulking does seem to be connected with a decrease in the

adsorptive power of the sludge and must, therefore, be associated with some change in the physical state of the zooglycal matrix about the bacterial cells.

The sudden appearance of carbohydrates is not necessarily the only possible agency by which activated sludges can be "shocked" into a bulking condition. The majority of the cases of bulking in activated sludges are those not involving the presence of carbohydrates in the sewage. The activated sludge system involves the interaction of three factors—sludge, food, and oxygen. A sudden change in the quality of any one or more of these factors may serve to induce bulking in the sludge. Ingols and Heukelekian (4) have shown recently that bulking can be induced in sludges, with reduced aeration rates, by feeding them such materials as propionate, butyrate, glycerol, and even peptone. Hence, glucose is not the only material that may act to give rise to bulking of sludges. Any sudden appreciable change in the form of food material may be expected to "shock" a sludge into a bulking condition.

In a previous paper (1) these same investigators pointed out several situations productive of bulking in sludges being fed sewage only. They showed that, with a given sewage and a given sludge concentration, bulking may result from a sudden change in rate of air supply, particularly if the quantity of air has been appreciably decreased; also, that with a given sludge concentration and aeration rate, bulking can be induced by an abnormally strong sewage. They pointed out that frequent additions of a low B. O. D. sewage will not affect the sludge, but the same total amount of B. O. D. added in the form of a few additions of a very strong sewage will result in bulking. Higher temperatures, accelerating the rate of interaction of sludge, food, and oxygen will tend to accentuate the unbalanced condition; hence bulking is encountered more frequently under summer conditions than under winter conditions.

Activated sludges are very efficient agents for the biological purification of sewages and other wastes. After having been developed under given uniform conditions from a given type of influent, the sludges will have developed properties permitting them to handle that sewage most efficiently under those same conditions. If the conditions of plant operation are gradually changed, the sludge is capable of adapting itself to the new conditions. But, if the changes in conditions are sudden and large, the sludge undergoes a biophysical strain in meeting this new situation. When this "strain" is severe the properties of the floc are changed and bulking is the result. Consequently, to insure the most efficient operation of a plant, the operator must control as far as possible sudden changes in conditions which might upset or strain the sludge. The operator, unfortunately, is usually decidedly limited in preventing such changes by plant design.

In fact, most plants are not designed with sufficient flexibility to enable the plant operator to make proper adjustments when emergency conditions causing bulking arise. At some plants bulking almost always persists, indicating a continuous lack of equilibrium in the sludge, sewage, and air factors. This shows that a sludge cannot become adjusted to all situations. In such cases the factor causing the continuous "shock" must be found and corrected. No doubt there are constituents in some industrial wastes to which activated sludge would never become conditioned. Wastes containing the simple carbohydrates are not in this class. As shown, with proper food balance and sufficient aeration, activated sludge becomes conditioned to them and recovery from bulking takes place. In these cases a larger quantity of sludge with a lower ash content can be expected than would be obtained from domestic sewage.

We do not believe that there is any reason to differentiate bulking into two classes, carbohydrate *Sphaerotilus* bulking and sewage bulking. All bulking conditions that occur in activated sludge arise from a biophysical strain of the sludge due to sudden changes in operating conditions. Some of the vectors capable of producing such strains are now known to a certain extent. Undoubtedly, there are many more not yet discovered or appreciated. Most of the cases of bulking are probably caused by improper plant design and operating conditions. Others are due to the sudden appearance of abnormal materials or abnormal quantities of common materials in sewage. The former cases can be avoided; the latter can not. But even here, plant operators expecting the abnormal appearance of certain industrial wastes (for example canning wastes) should be on the lookout for them and make changes in operation procedures, if possible, to meet the expected conditions.

SUMMARY

Our strains of *Sphaerotilus natans* were found to be obligate aerobes, being similar to the zooglear bacteria in this respect. They differed from the zooglear in their greater variability in viability and metabolism. Compared to zooglear bacteria *Sphaerotilus natans* must be considered a delicate organism because of its sensitivity to variations in environmental factors. Its growth rate in pure culture in a glucose-peptone medium increases with aeration rate to rates considerably higher than those ordinarily used in practice. Its optimum pH range is from 6 to 9 and it is particularly sensitive to pH values below 5. The optimum temperature range is about 30° C. and growth practically ceases at 10° C. even at optimum conditions of other environmental factors.

Experiments to induce bulking indicated that bulking was a response of sludge organisms (zoogloal bacteria and probably others) to a sudden disturbance in biological equilibrium. The three primary factors involved include the sludge, food, and rate of oxygen supply. Variations in one or more of these factors may produce the disturbance causing bulking. This disturbance affects primarily the biophysical character of the matrix as indicated by a reduction in short time adsorption capacity and by the formation of a light non-compact floc. The disturbance does not immediately affect the oxidizing capacity of the floc. The phenomenon can therefore best be described as a biophysical response to a sudden change in biological equilibrium. The appearance of *Sphaerotilus natans* is not a primary cause of bulking. The disturbance to which the sludge floc responds to produce bulking in certain instances also produces *Sphaerotilus* growths. In such cases the interweaving of the *Sphaerotilus natans* filaments with the light floc accentuates the condition.

ACKNOWLEDGMENT

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LEPROSY: COMPLEMENT FIXATION WITH GAEHTGENS' SPIROCHETE ANTIGEN COMPARED WITH STANDARD WASSERMANN AND KAHN TESTS

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Capelli (1) in 1939 performed complement fixation tests on the serums of 24 leprous patients, employing Gaehtgens' phenolized cultures of *Treponema pallidum* (palligen) as antigen, and compared the results with those obtained with the Wassermann procedure and the Meinicke flocculation test with the same serums. He reported that with Gaehtgens' test none of the serums gave a positive (4 or 3 plus) result with the exception of that from one case which was considered to be syphilitic. The results were partially positive (1 or 2 plus) with 22 percent and negative with 78 percent of the serums. The Wassermann reactions were positive with 66 percent, partial with 17 percent, and negative with 17 percent of the serums. The Meinicke reactions were positive with 39 percent, partial with 22 percent, and negative with 39 percent of the serums. In performing the spirochete complement fixation test Capelli followed Gaehtgens' method which is essentially a "one-tube test" employing only one dose of the serum to be tested.

It was believed that the results obtained by Capelli were of sufficient significance to warrant a comparative study of the results obtained with the spirochete and Wassermann complement fixation and the Kahn flocculation reactions with serums of a larger number of leprous patients.

Each test was performed on a single specimen of serum obtained from 94 patients in Kalihi Hospital, Honolulu, who were considered nonsyphilitic after careful study, including detailed histories and complete physical examinations.

Badger et al. (2, 3) noted that changes in serum reactions were correlated with changes in the clinical manifestations of the disease and that positive results were obtained more frequently with the serums of the nodular-infiltrated than with the maculo-anesthetic cases. In accordance with his results the cases employed in this study

have been divided into three groups—maculo-anesthetic bacteriologically negative, maculo-anesthetic bacteriologically positive, and nodular.

Gaetgens' original procedure¹ was not followed exactly because it was considered more desirable to use the technique of the standard Wassermann (Kolmer) test, merely substituting the palligen for the Kolmer² antigen. In determining the proper antigenic dose of palligen it was found that Gaetgens' recommended dose¹ (0.25 cc. of equal parts of stock palligen and 0.3 percent phenolized physiological salt solution) proved neither hemolytic nor anticomplementary and contained approximately 40 antigenic units when used in the Kolmer technique. This dose was used in our entire series of tests. Serum dilutions of 1:5, 1:10, and 1:20 were examined and the standard complement fixation procedure was closely followed. The final result of each serum specimen was obtained by totaling or summarizing the results of the three serum dilutions according to the usual method of quantitative reporting.

TABLE 1.—Results of the spirochete complement fixation, Wassermann and Kahn tests on the serums of 94 nonsyphilitic leprous patients

Type of case	Positive reaction +++ and +++						Doubtful reaction ++ and +						Negative reaction					
	Spirochete complement fixation		Wassermann		Kahn		Spirochete complement fixation		Wassermann		Kahn		Spirochete complement fixation		Wassermann		Kahn	
	Number	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Percent
Maculo-anesthetic, bacteriologically negative, 17 cases.	11	5.9	0	0	0	0	0	0	0	0	0	0	16	94.1	17	100.0	17	100.0
Maculo-anesthetic, bacteriologically positive, 30 cases.	11	3.3	3	10.0	5	16.6	2	6.7	0	0	0	0	27	90.0	27	90.0	25	83.4
Nodular, 47 cases	5	10.5	33	70.0	31	66.1	4	8.5	2	4.3	5	10.5	38	81.0	12	25.7	11	23.4
Total, 94 cases	7	7.4	36	38.3	36	38.3	6	6.4	2	2.2	5	5.3	81	86.2	56	59.5	53	56.4

¹ With these serums the results obtained with the Wassermann and Kahn tests were negative.

² The results obtained with each of these serums with the Wassermann and Kahn tests were also positive.

The results obtained are illustrated in the accompanying table. It is apparent that fewer positive results were obtained with the spirochete complement fixation test than with either the Wassermann or Kahn test³—7.4 percent with the former and 38.3 percent

¹ The antigen (lot No. 119) was secured from the Saxon Serum Works, Dresden, Germany, and is sold under the trade name "Palligen." The Gaetgens procedure accompanied the antigen.

² The Kolmer and Kahn antigens and the antishcep hemolysin were commercial products.

³ The results of our Wassermann and Kahn tests were frequently checked in two other laboratories, Queen's Hospital and Territorial Board of Health.

with each of the latter. It also will be noted that, as with the Wassermann and Kahn tests, more positive results were obtained with the serums of the nodular cases than with those of the maculo-anesthetic cases.

CONCLUSIONS

When examined with the spirochete complement fixation test, the serums of nonsyphilitic leprous patients exhibit a tendency toward falsely positive results, although to a lesser degree than with the Wassermann and Kahn tests.

With the spirochete complement fixation test as well as with the Wassermann and Kahn tests, more positive results are obtained with the serums of the nodular type than with serums of the maculo-anesthetic type.

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PREVALENCE OF COMMUNICABLE DISEASES IN THE UNITED STATES

July 13–August 9, 1941

The accompanying table summarizes the prevalence of nine important communicable diseases, based on weekly telegraphic reports from State health departments. The reports from each State are published in the PUBLIC HEALTH REPORTS under the section "Prevalence of disease." The table gives the number of cases of these diseases for the 4-week period ended August 9, 1941, the number reported for the corresponding period in 1940, and the median number for the years 1936–40.

Number of reported cases of 9 communicable diseases in the United States during the 4-week period July 13–August 9, 1941, the number for the corresponding period in 1940, and the median number of cases reported for the corresponding period, 1936–40

Division	Current period	1940	5-year median	Current period	1940	5-year median	Current period	1940	5-year median
	Diphtheria			Influenza ¹			Measles ²		
United States.....	609	640	1,111	2,715	1,476	1,069	12,170	10,086	8,294
New England.....	17	16	21	3	3	3	1,279	1,927	899
Middle Atlantic.....	68	74	138	13	13	20	3,336	3,213	2,631
East North Central.....	99	110	205	73	93	93	2,607	2,618	2,328
West North Central.....	51	63	69	26	11	71	467	373	265
South Atlantic.....	123	124	219	707	525	324	2,269	400	535
East South Central.....	50	50	126	72	59	97	411	372	210
West South Central.....	105	91	148	1,370	636	261	605	362	231
Mountain.....	56	51	51	161	86	66	407	345	345
Pacific.....	40	61	82	290	49	55	771	476	763
	Meningococcus meningitis			Pollomyelitis			Scarlet fever		
United States.....	116	106	151	1,296	716	716	2,714	2,985	3,508
New England.....	6	5	6	27	7	16	274	157	205
Middle Atlantic.....	31	17	31	130	19	34	588	796	740
East North Central.....	13	11	21	146	183	183	779	939	1,157
West North Central.....	8	13	13	40	127	69	239	256	396
South Atlantic.....	22	21	29	490	65	65	228	244	247
East South Central.....	20	20	24	389	42	42	169	147	149
West South Central.....	7	8	15	32	89	42	103	103	159
Mountain.....	5	4	6	12	41	22	80	100	150
Pacific.....	4	7	14	30	143	130	204	243	255
	Smallpox			Typhoid and paratyphoid fever			Whooping cough ³		
United States.....	29	108	239	1,199	1,481	2,058	16,099	13,822	³ 14,614
New England.....	0	0	0	23	33	39	1,123	945	929
Middle Atlantic.....	0	0	0	164	122	141	2,611	3,124	4,526
East North Central.....	10	20	66	136	113	220	4,156	3,553	4,423
West North Central.....	9	45	81	62	113	118	1,169	760	760
South Atlantic.....	2	1	1	264	284	493	2,351	1,691	1,891
East South Central.....	0	8	4	187	185	427	503	591	571
West South Central.....	3	6	6	264	513	541	1,037	1,163	1,016
Mountain.....	4	20	36	53	45	55	1,171	582	582
Pacific.....	1	8	31	46	73	70	1,989	1,413	1,168

¹ Mississippi, New York, and Pennsylvania excluded; New York City included.

² Mississippi excluded.

³ Three-year (1938–40) median.

DISEASES ABOVE MEDIAN PREVALENCE

Influenza.—Influenza still maintains a relatively high level. There were 2,715 cases reported for the 4 weeks ended August 9, which was about 1.8 times the number reported for the corresponding period in 1940 and more than 2.6 times the 1936–40 median figure for the period. The North Atlantic, North Central, and East South Central regions reported a comparatively low incidence, but in the South Atlantic, West South Central, Mountain, and Pacific regions the numbers of cases were relatively high. The lowest incidence of this disease is usually reached during this period of the year and while the disease has followed the usual trend, the number of cases occurring during the

current period is the highest recorded for this period in the 13 years for which these data are available.

Measles.—The incidence of measles was also the highest in recent years. While the number of cases dropped from approximately 45,000 during the preceding 4-week period to approximately 12,000 during the current 4-week period, the number of cases was almost 25 percent in excess of the 1940 incidence and almost 50 percent in excess of the 1936–40 average incidence for the corresponding period. Each section of the country contributed to the high incidence of this disease but the largest numbers of cases were reported from the regions along the Atlantic coast and the East North Central regions. In the South Atlantic region the number of cases was more than 4 times the normal seasonal incidence.

Poliomyelitis.—The number of cases of poliomyelitis rose from 415 cases for the preceding 4-week period to 1,296 for the 4 weeks ended August 9. The current figure was 1.8 times the incidence during the corresponding period in 1940 (716 cases), which figure also represents the average incidence for the period. The current outbreak of this disease started in June in the South Atlantic and East South Central regions and of the total number of cases for the current period 312 were reported from Georgia, 233 from Alabama, 80 from Tennessee, and 69 from Florida; more than one-half of all of the reported cases occurred in those 4 States. During the latter part of the current period, the States in the Middle Atlantic region reported rather sharp increases in the number of cases, with minor rises in the New England States. Other regions have shown no signs of anything other than the normal rise expected at this season of the year; and for the current period in these regions the disease is considerably less prevalent than it was in 1940 and is well below the seasonal expectancy. For the country as a whole the current incidence has been exceeded only twice since 1931—in 1937, when the cases for this period totaled 1,594, and in 1935, when a total of 1,433 cases was recorded for the period.

Whooping cough.—The number of cases of whooping cough was considerably above the average seasonal level, approximately 16,000 cases as compared with a 1938–40 median of approximately 14,600 cases. The Middle Atlantic, East North Central, and East South Central regions reported a relatively low incidence, but in all other regions the cases were considerably in excess of the average seasonal incidence.

DISEASES BELOW MEDIAN PREVALENCE

Diphtheria.—For the 4 weeks ended August 9 there were 609 cases of diphtheria reported, as compared with 640, 1,030, and 1,288 for the corresponding period in 1940, 1939, and 1938, respectively. In all regions except the Mountain the number of cases was lower than the

1936-40 median incidence for this period. The downward trend of this disease has been unbroken during this period in the 13 years for which these data are available; during the corresponding period in 1929, 3,520 cases were reported.

Meningococcus meningitis.—Due largely to an increase over last year in the number of cases of this disease in the Middle Atlantic region, the incidence (116 cases) for the country as a whole was about 10 percent in excess of the 1940 figure for this period. The number was, however, less than 80 percent of the (1936-40) average seasonal incidence, with the number of cases in the North Atlantic and Mountain regions standing at the expected seasonal level and all other regions reporting a relatively low incidence.

Scarlet fever.—The incidence of scarlet fever was also relatively low, the number of cases (2,714) being about 90 percent of last year's figure and less than 80 percent of the 1936-40 median incidence for this period. A few more cases than might normally be expected were reported from the New England and East South Central regions, but in all other regions the incidence was comparatively low.

Smallpox.—The reported cases (29) of smallpox dropped considerably below the number reported for the corresponding period last year, which number (108) was the lowest on record for this period. No cases were reported from the North Atlantic and East South Central regions, 10 occurred in the East North Central region, 9 in the West North Central region, and the remaining 10 cases were scattered over the remainder of the country.

Typhoid fever.—Typhoid fever was considerably less prevalent than it was in 1940 and the incidence reached a new low level for this period. The number of reported cases was 1,199, as compared with 1,481, 2,001, and 2,022 for the corresponding period in 1940, 1939, and 1938, respectively. In the Middle Atlantic region the number of cases was slightly above the seasonal average and in the Mountain region the incidence was about normal, but all other regions reported a relatively low incidence.

MORTALITY, ALL CAUSES

The average mortality rate from all causes in large cities for the 4 weeks ended August 9, based on data received from the Bureau of the Census, was 10.6 per 1,000 inhabitants (annual basis). The average rate for this period in the 3 preceding years was 10.5 per 1,000.

DEATHS DURING WEEK ENDED AUGUST 16, 1941

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

	Week ended Aug. 16, 1941	Correspond- ing week, 1940
Data from 87 large cities of the United States:		
Total deaths.....	7,294	6,920
Average for 3 prior years.....	7,237	
Total deaths, first 33 weeks of year.....	284,359	284,994
Deaths per 1,000 population, first 33 weeks of year, annual rate.....	12.1	12.1
Deaths under 1 year of age.....	477	458
Average for 3 prior years.....	486	
Deaths under 1 year of age, first 33 weeks of year.....	17,358	16,601
Data from industrial insurance companies:		
Policies in force.....	64,418,462	64,932,518
Number of death claims.....	10,925	12,001
Death claims per 1,000 policies in force, annual rate.....	8.8	9.7
Death claims per 1,000 policies, first 33 weeks of year, annual rate.....	9.8	10.0

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

REPORTS FROM STATES FOR WEEK ENDED AUGUST 23, 1941

Summary

A total of 611 cases of poliomyelitis was reported for the current week, as compared with 549 for last week—a smaller numerical increase than that recorded for either of the preceding two weeks. Most of the current increase occurred in the 3 Middle Atlantic States—New York, New Jersey, and Pennsylvania. The South Atlantic, East South Central, and Middle Atlantic States reported the largest numbers of cases and the highest incidence rates for the current week, and have recorded the highest rates this year to date. These States reported 75 percent of the current total.

The following 11 States reported 15 or more cases (last week's figures in parentheses): Pennsylvania, 82 (45); Alabama, 78 (82); Georgia, 74 (69); New York, 66 (49); Ohio, 44 (37); Tennessee, 39 (37); New Jersey, 25 (17); Kentucky, 25 (15); Illinois, 23 (18); Maryland, 21 (16); and California, 16 (5). Twelve States reported 15 or more cases last week.

More cases of poliomyelitis have been reported in the United States this year to date (3,433) than for the corresponding period of any year since 1937, when 4,250 cases were reported.

North Dakota reported 120 cases of infectious encephalitis (340 last week), Minnesota 95 (121 last week), South Dakota 38 (44 last week), and Colorado 20 (epidemic or lethargic) (32 last week). The fatality rate in North Dakota has been about 10 percent.

The fatal human case of plague reported in California during the week ended August 16 occurred in Siskiyou County.¹

Ninety-eight cases of endemic typhus fever were reported, as compared with 166 last week, when 123 cases were reported in Texas (104 in Lavaca County). For the current week, Georgia reported 44 cases, Texas 23, Louisiana 10, and Alabama 9.

Of 26 cases of Rocky Mountain spotted fever, only 4 occurred in the Rocky Mountain area.

The death rate for the current week for 88 large cities was 9.9 per 1,000 population, as compared with 10.2 last week and with a 3-year (1938-40) average of 9.8.

¹ See p. 1767.

Telegraphic morbidity reports from State health officers for the week ended August 23, 1941, and comparison with corresponding week of 1940 and 5-year median

In these tables a zero indicates a definite report, while leaders imply that, although none were reported, cases may have occurred.

Division and State	Diphtheria			Influenza			Measles			Meningitis, meningococcus		
	Week ended—		Median 1936-40	Week ended—		Median 1936-40	Week ended—		Median 1936-40	Week ended—		Median 1936-40
	Aug. 23, 1941	Aug. 24, 1940		Aug. 23, 1941	Aug. 24, 1940		Aug. 23, 1941	Aug. 24, 1940		Aug. 23, 1941	Aug. 24, 1940	
NEW ENG.												
Maine.....	1	1	0				9	2	6	0	0	0
New Hampshire.....	0	0	0				0	0	0	0	0	0
Vermont.....	0	0	0				12	1	2	0	0	0
Massachusetts.....	4	1	2				73	70	36	0	2	1
Rhode Island.....	3	0	0				0	10	5	0	0	0
Connecticut.....	0	0	0				24	4	8	1	0	0
MID ATL.												
New York ¹	7	7	9	10	14	12	90	138	93	2	3	3
New Jersey.....	1	5	6	2		6	31	60	37	0	0	1
Pennsylvania ²	8	2	15				92	107	39	0	1	2
E. NO. CEN.												
Ohio ³	4	12	12	2	8	4	28	15	15	1	2	2
Indiana ³	1	0	7	7		4	1	2	4	2	0	0
Illinois.....	9	8	15	2		4	24	35	35	3	2	2
Michigan ⁴	0	5	6		6	1	27	83	24	1	1	0
Wisconsin.....	1	0	1	2	11	15	76	82	24	1	1	1
W. NO. CEN.												
Minnesota.....	3	0	1	2	5	1	3	2	2	0	0	0
Iowa ²	0	5	2		3		5	7	7	0	2	1
Missouri ³	5	9	6		1	7	7	3	2	0	2	1
North Dakota.....	0	8	2	18			18	0	0	0	1	0
South Dakota.....	7	0	0				2	0	0	0	0	0
Nebraska.....	1	0	1				0	1	3	0	1	0
Kansas.....	2	6	3	2		1	11	9	9	2	1	1
SO. ATL.												
Delaware.....	0	0	0				1	0	0	0	0	0
Maryland ^{3,4}	1	8	3	3	3	2	4	3	10	2	0	0
Dist. of Col.....	0	1	2				10	2	2	0	0	0
Virginia ³	16	2	18	40	83		22	27	16	4	0	0
West Virginia ^{3,4}	6	1	6	24	13	13	45	1	2	0	1	1
North Carolina ³	14	9	31				14	4	5	0	0	1
South Carolina ¹	22	8	9	56	129	112	55	16	5	0	0	0
Georgia ¹	11	10	22	8	1		37	0		0	0	0
Florida ¹	2	3	3	2		1	4	2	2	0	0	0
E. SO. CEN.												
Kentucky.....	3	7	9			3	6	15	4	0	0	0
Tennessee.....	10	2	14	11	6	9	15	23	14	0	1	1
Alabama ¹	11	10	15	7	1	4	4	26	17	1	2	1
Mississippi ^{1,4}	14	3	15							0	0	0
W. SO. CEN.												
Arkansas.....	10	3	8	2	1	5	12	10	5	1	0	0
Louisiana ^{1,3}	5	10	10		2		1	2	2	0	3	1
Oklahoma.....	3	2	7	19	20	10	7	3	3	0	0	0
Texas ^{1,4}	14	11	23	267	61	37	163	24	16	1	1	1
MOUNTAIN												
Montana ²	1	2	1				3	5	5	0	0	0
Idaho.....	0	0	0				0	0	1	0	0	0
Wyoming.....	0	0	0				3	3	1	0	0	0
Colorado ³	10	7	7	19	2		14	5	3	0	0	1
New Mexico.....	1	2	2		1		4	1	5	0	0	0
Arizona.....	1	1	2	15	12	12	20	12	3	0	0	0
Utah ^{3,4}	0	1	1	9			6	9	6	0	0	0
Nevada.....	0						2			0		
PACIFIC												
Washington.....	0	0	2				1	9	13	1	0	0
Oregon.....	5	1	1	6	4	4	8	15	7	0	2	0
California.....	3	8	17	16	4	11	74	31	49	1	0	0
Total.....	220	181	339	539	384	356	1,068	879	745	24	29	29
34 weeks.....	7,846	9,046	14,082	600,411	169,606	152,006	831,515	229,371	270,548	1,440	1,187	2,188

See footnotes at end of table.

Telegraphic morbidity reports from State health officers for the week ended August 23, 1941, and comparison with corresponding week of 1940 and 5-year median—Con.

Division and State	Polymyelitis			Scarlet fever			Smallpox			Typhoid and paratyphoid fever		
	Week ended—		Median 1936-40	Week ended—		Median 1936-40	Week ended—		Median 1936-40	Week ended—		Median 1936-40
	Aug. 23, 1941	Aug. 24, 1940		Aug. 23, 1941	Aug. 24, 1940		Aug. 23, 1941	Aug. 24, 1940		Aug. 23, 1941	Aug. 24, 1940	
NEW ENG.												
Maine	2	0	0	2	1	2	0	0	0	0	0	2
New Hampshire	0	0	0	2	0	0	0	0	0	0	1	1
Vermont	0	0	0	2	1	1	0	0	0	0	1	0
Massachusetts	8	4	4	36	21	21	0	0	0	1	1	3
Rhode Island	4	1	1	4	0	0	0	0	0	0	1	1
Connecticut	7	0	0	6	2	5	0	0	0	1	3	3
MID. ATL.												
New York ¹	66	14	14	35	57	57	0	0	0	18	18	18
New Jersey	25	4	4	15	18	17	0	0	0	7	8	8
Pennsylvania ²	82	7	7	30	31	43	0	0	0	18	15	25
E. NO. CEN.												
Ohio ³	44	46	8	28	48	48	0	0	0	7	18	17
Indiana ³	7	79	1	8	17	20	0	1	1	2	1	7
Illinois	23	21	15	38	43	51	0	0	1	23	12	23
Michigan ⁴	6	98	31	27	30	40	1	1	1	7	4	7
Wisconsin	2	12	8	28	38	38	0	2	1	0	2	2
W. NO. CEN.												
Minnesota	14	8	8	8	14	16	0	2	0	0	0	1
Iowa ³	2	73	4	5	14	15	0	1	2	3	8	8
Missouri ³	0	18	2	6	14	15	0	0	0	7	20	22
North Dakota	0	2	0	0	0	5	0	1	1	0	0	1
South Dakota	0	4	2	2	1	7	0	0	0	0	0	1
Nebraska	0	15	2	3	3	3	1	0	0	0	1	1
Kansas	1	42	1	16	20	19	0	0	0	0	5	6
SO. ATL.												
Delaware	2	0	0	1	2	0	0	0	0	1	1	1
Maryland ^{3,4}	21	1	1	31	3	7	0	0	0	11	6	13
Dist. of Col.	6	0	1	4	3	3	0	0	0	2	5	3
Virginia ³	9	6	4	11	12	11	0	0	0	10	5	20
West Virginia ^{3,4}	1	46	0	13	20	20	0	0	0	11	6	10
North Carolina ³	4	4	4	11	13	16	0	0	0	7	8	20
South Carolina ¹	8	0	1	5	5	5	0	2	0	6	24	14
Georgia ¹	74	0	2	14	6	10	0	0	0	28	19	26
Florida ¹	14	2	1	2	3	2	0	0	0	7	3	3
E. SO. CEN.												
Kentucky	25	18	4	23	13	16	1	0	0	16	15	31
Tennessee	39	1	2	13	10	14	0	0	0	15	21	21
Alabama ¹	78	0	1	11	14	14	0	0	0	5	13	17
Mississippi ^{1,4}	5	5	5	4	5	5	0	0	0	12	11	9
W. SO. CEN.												
Arkansas	1	1	1	2	4	9	0	0	0	13	26	26
Louisiana ³	7	10	2	3	2	2	0	0	0	9	25	25
Oklahoma	1	14	1	5	7	7	0	1	0	5	25	24
Texas ^{1,4}	2	14	10	18	14	16	0	0	0	32	59	50
MOUNTAIN												
Montana ³	0	15	1	9	2	6	0	0	2	1	0	1
Idaho	0	2	0	1	2	3	0	0	1	1	0	1
Wyoming	0	0	0	0	2	3	0	0	0	0	4	0
Colorado ³	1	2	2	9	5	7	0	2	1	1	3	5
New Mexico	0	2	1	1	2	2	0	0	0	3	4	4
Arizona	0	1	1	1	1	1	0	0	0	0	0	5
Utah ^{3,4}	1	4	1	2	5	5	0	0	0	2	1	2
Nevada	0			0			0			0		
PACIFIC												
Washington	0	13	2	7	14	12	0	0	1	9	0	4
Oregon	3	1	1	11	5	5	0	0	1	4	2	3
California	16	13	13	45	41	57	0	0	1	3	9	9
Total	611	623	391	558	588	697	3	13	34	308	412	571
34 weeks	3,433	2,695	2,530	92,453	119,475	137,851	1,193	1,971	8,046	4,804	5,405	7,584

See footnotes at end of table.

Telegraphic morbidity reports from State health officers for the week ended, August 23, 1941, and comparison with corresponding week of 1940—Continued

Division and State	Whooping cough		Division and State	Whooping cough	
	Week ended—			Week ended—	
	Aug. 23, 1941	Aug. 24, 1940		Aug. 23, 1941	Aug. 24, 1940
NEW ENG.			SO. ATL.—continued		
Maine.....	13	16	South Carolina ¹	53	25
New Hampshire.....	1	0	Georgia ¹	20	11
Vermont.....	14	9	Florida ¹	13	1
Massachusetts.....	124	116	E. SO. CEN.		
Rhode Island.....	15	7	Kentucky.....	51	66
Connecticut.....	44	38	Tennessee.....	44	30
MID. ATL.			Alabama ¹	12	55
New York ¹	253	247	Mississippi ^{1,4}	-----	-----
New Jersey.....	116	112	W. SO. CEN.		
Pennsylvania ²	193	400	Arkansas.....	7	18
E. NO. CEN.			Louisiana ^{1,3}	12	57
Ohio ²	221	263	Oklahoma.....	6	8
Indiana ²	18	11	Texas ^{1,4}	92	188
Illinois.....	213	156	MOUNTAIN		
Michigan ⁴	182	215	Montana ²	21	9
Wisconsin.....	208	102	Idaho.....	17	8
W. NO. CEN.			Wyoming.....	15	0
Minnesota.....	53	40	Colorado ³	108	13
Iowa ²	29	22	New Mexico.....	54	4
Missouri ²	4	42	Arizona.....	17	5
North Dakota.....	18	21	Utah ^{2,4}	48	26
South Dakota.....	18	3	Nevada.....	1	-----
Nebraska.....	4	3	PACIFIC		
Kansas.....	58	26	Washington.....	52	23
SO. ATL.			Oregon.....	17	21
Delaware.....	1	0	California.....	267	258
Maryland ^{3,4}	28	90	Total.....		
Dist. of Col.....	23	6	2,971		2,965
Virginia ²	57	59	149,750		110,137
West Virginia ^{3,4}	29	62	34 weeks.....		
North Carolina ²	107	82			

¹ Typhus fever, week ended August 23, 1941, 98 cases as follows: New York, 2; South Carolina, 3; Georgia, 44; Florida, 5; Alabama, 9; Mississippi, 2; Louisiana, 10; Texas, 23.

² New York City only.

³ Rocky Mountain spotted fever, week ended August 23, 1941, 26 cases as follows: Pennsylvania, 2; Ohio, 3; Indiana, 1; Iowa, 2; Missouri, 2; Maryland, 5; Virginia, 4; West Virginia, 1; North Carolina, 1; Louisiana, 1; Montana, 1; Colorado, 1; Utah, 2.

⁴ Period ended earlier than Saturday.

HUMAN CASE OF PLAGUE IN SISKIYOU COUNTY, CALIF.

A fatal case of human plague was reported in Siskiyou County, Calif., on August 11, 1941, with onset on August 6 or 7, and death on August 9. The case occurred in a 5-year-old boy living 1 mile north-west of Mount Shasta City. The diagnosis was confirmed by animal inoculation and the isolation of pure cultures.

The case occurred about 50 miles from the locality in which a fatal case was reported in the same county in June.¹ It represents a new focus of the infection in California and indicates widespread rodent plague in the county.

¹ Public Health Reports, July 4, 1941, p. 1400.

PLAGUE INFECTION IN A GROUND SQUIRREL IN HARNEY COUNTY, OREG.

Under date of August 14, 1941, plague infection was reported found, upon examination at the laboratory in San Francisco, Calif., in tissue from a ground squirrel, *C. oregonus*, shot July 30 at Fish Lake, 80 miles southeast of Burns, Harney County, Oreg.

WEEKLY REPORTS FROM CITIES

City reports for week ended August 9, 1941

This table lists the reports from 135 cities of more than 10,000 population distributed throughout the United States, and represents a cross section of the current urban incidence of the diseases included in the table.

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	0	0	0	0	0	0	1	21
New Hampshire:											
Concord	0		0	0	1	0	0	1	0	0	11
Manchester	0		0	0	0	1	0	0	0	0	7
Nashua	0		0	0	1	0	0	0	0	0	6
Vermont:											
Barre	0		0	0	0	0	0	0	0	0	2
Burlington	0		0	0	0	0	0	0	0	1	10
Rutland	0		0	0	0	0	0	0	0	0	8
Massachusetts:											
Boston	1		0	15	5	18	0	5	5	34	167
Fall River	0		0	2	2	4	0	0	0	2	24
Springfield	0		0	4	0	6	0	1	0	2	32
Worcester	0		0	0	1	1	0	1	0	17	30
Rhode Island:											
Providence	1		0	2	1	2	0	1	0	25	52
Connecticut:											
Bridgeport	0		0	4	0	0	0	0	0	0	24
Hartford	0		0	1	0	1	0	1	0	0	26
New Haven	0		0	3	0	1	0	0	0	9	28
New York:											
Buffalo	0		0	2	5	0	0	2	0	9	110
New York	12	1	1	35	34	15	0	70	9	130	1,285
Rochester	0		0	1	0	0	0	0	0	3	65
Syracuse	0		0	2	0	0	0	0	0	9	39
New Jersey:											
Camden	0		0	0	1	0	0	0	0	7	25
Newark	0		0	15	6	4	0	5	0	21	89
Trenton	0	1	0	1	0	0	0	2	0	0	29
Pennsylvania:											
Philadelphia	0		0	1	12	7	0	17	2	31	398
Pittsburgh	0		2	9	4	3	0	7	2	36	138
Reading	0		0	0	1	0	0	0	0	0	18
Scranton	0			1		0	0		0	1	
Ohio:											
Cincinnati	0		0	9	0	2	0	4	1	9	115
Cleveland	0	1	1	0	4	11	0	13	0	73	186
Columbus	0		0	2	0	0	0	3	0	18	70
Toledo	0		0	13	3	2	0	1	0	39	73
Indiana:											
Anderson	0		0	0	0	0	0	0	0	0	9
Fort Wayne	0		0	0	3	0	0	0	0	0	30
Indianapolis	0		0	5	4	2	0	7	0	11	90
Muncie	0		0	0	1	0	0	2	0	0	15
South Bend	0		0	0	0	0	0	0	0	0	25
Terre Haute	1		0	0	1	0	0	0	0	0	22
Illinois:											
Alton	0		0	0	0	0	0	0	0	0	8
Chicago	7	2	1	9	20	9	0	37	1	12	633
Elgin	1		0	0	0	0	0	0	0	4	11
Moline	0		0	0	0	0	0	0	0	2	9
Springfield	0		0	12	2	0	0	0	0	0	18
Michigan:											
Detroit	0		0	20	7	9	0	15	0	113	220
Flint	0		0	2	4	0	0	0	0	2	23
Grand Rapids	0		0	4	0	1	0	0	0	3	29
Wisconsin:											
Kenosha	0		0	0	0	1	0	0	0	0	7
Madison	0		0	4	0	0	0	0	0	1	9
Milwaukee	0		0	35	1	14	0	3	0	122	96

City reports for week ended August 9, 1941—Continued

State and city	Influenza		Measles cases	Pneumonia deaths	Scarlet fever cases	Smallpox cases	Tuberculosis deaths	Typhoid fever cases	Whooping cough cases	Deaths, all causes
	Cases	Deaths								
Wisconsin—Con.										
Racine.....	0	0	13	0	2	0	1	0	8	17
Superior.....	0	0	5	0	0	0	0	0	6	10
Minnesota:										
Duluth.....	0	0	0	0	0	0	0	0	11	14
Minneapolis.....	0	0	2	2	2	0	1	0	16	101
St. Paul.....	0	0	2	0	0	0	2	0	16	54
Iowa:										
Cedar Rapids.....	0		1		1	0		0	0	
Davenport.....	0		1		0	0		0	0	
Des Moines.....	0		0		0	0		0	14	26
Sioux City.....	1		0		0	0		0	16	
Waterloo.....	0		1		0	0		0	4	
Missouri:										
Kansas City.....	0	0	3	4	3	0	5	0	4	91
St. Joseph.....	0	0	0	2	0	0	0	0	0	22
St. Louis.....	0	0	6	6	2	0	7	1	9	173
North Dakota:										
Grand Forks.....	1		1		0	0		0	0	
Minot.....	0	0	4	0	0	0	0	0	1	7
South Dakota:										
Aberdeen.....	0		0		0	0		0	0	
Sioux Falls.....	0		0		0	0		0	0	6
Nebraska:										
Lincoln.....	0		4		1	0		0	4	
Omaha.....	0	0	5	1	0	0	0	0	2	44
Kansas:										
Lawrence.....	0	0	0	0	0	0	0	0	6	3
Topeka.....	0	0	0	0	0	0	2	0	15	26
Wichita.....	0	0	0	6	0	0	0	1	3	27
Delaware:										
Wilmington.....	0	0	1	4	0	0	1	0	0	0
Maryland:										
Baltimore.....	0	0	43	7	5	0	12	1	59	213
Cumberland.....	0	0	1	0	0	0	0	0	0	11
Frederick.....	0	0	1	0	0	0	0	0	0	4
Dist. of Col.										
Washington.....	1	0	11	11	3	0	9	0	21	139
Virginia:										
Lynchburg.....	1	0	1	0	0	0	0	0	1	7
Norfolk.....	0	0	5	1	0	0	0	0	0	30
Richmond.....	1	0	2	3	0	0	2	0	0	71
Roanoke.....	0	0	0	0	1	0	0	0	0	13
West Virginia:										
Charleston.....	0	0	0	3	0	0	3	0	0	39
Huntington.....	1		0		0	0		0	0	
Wheeling.....	0	0	0	0	0	0	0	0	0	19
North Carolina:										
Gastonia.....	0		0		0	0		0	0	
Raleigh.....	0	0	2	0	1	0	0	0	12	11
Wilmington.....	0	0	0	1	0	0	0	0	11	11
Winston-Salem.....	0	0	4	0	0	0	2	1	2	
South Carolina:										
Charleston.....	0	1	0	1	1	0	0	2	0	26
Florence.....	0		0		0	0		0	1	
Greenville.....	0		0		0	0		1	1	10
Georgia:										
Atlanta.....	3	0	0	2	2	0	9	2	4	70
Brunswick.....	0	0	0	0	0	0	0	0	0	5
Savannah.....	0	0	0	0	0	0	0	0	4	27
Florida:										
Miami.....	0	1	1	6	4	0	2	1	11	37
St. Petersburg.....	0		0	0	0	0	0	4	0	15
Tampa.....	0		0	2	0	0	1	0	0	25
Kentucky:										
Ashland.....	0	0	0	0	1	0	0	0	0	8
Covington.....	0	0	0	0	1	1	0	0	0	14
Lexington.....	0	0	0	0	0	0	0	0	5	16
Louisville.....	1	1	0	3	7	2	0	2	10	56
Tennessee:										
Knoxville.....	0	4	0	0	1	0	3	1	1	29
Memphis.....	0	0	2	1	0	0	2	0	18	90
Nashville.....	0	0	1	5	0	0	6	1	10	52
Alabama:										
Birmingham.....	2	0	0	1	0	0	4	5	1	65
Mobile.....	0	2	0	2	0	0	1	0	0	19
Montgomery.....	0		0		1	0		0	0	
Arkansas:										
Fort Smith.....	0		0		0	0		0	1	
Little Rock.....	0		1	2	0	0	1	0	0	13

City reports for week ended August 9, 1941—Continued

State and city	Influenza		Measles cases	Pneumonia deaths	Scarlet fever cases	Small-pox cases	Tuberculosis deaths	Typhoid fever cases	Whooping cough cases	Deaths, all causes
	Cases	Deaths								
Louisiana:										
Lake Charles	0	0	0	0	0	0	0	0	0	8
New Orleans	0	0	0	6	0	0	10	1	15	119
Shreveport	0	0	0	0	0	0	4	0	0	24
Oklahoma:										
Oklahoma City	0	0	0	6	0	0	2	1	0	57
Tulsa	0	0	1	1	1	0	2	1	1	20
Texas:										
Dallas	2	0	7	1	0	0	1	0	1	76
Fort Worth	0	0	1	3	0	0	2	0	0	38
Galveston	0	0	0	0	0	0	1	0	0	20
Houston	1	1	0	2	0	0	5	0	1	68
San Antonio	0	5	3	0	6	0	11	0	0	74
Montana:										
Billings	0	0	0	0	1	0	0	0	3	9
Great Falls	0	0	0	0	0	0	0	0	3	11
Helena	0	0	0	0	0	0	0	0	1	3
Missoula	0	0	0	0	0	0	0	0	0	4
Idaho:										
Boise	0	0	0	0	0	0	0	0	0	9
Colorado:										
Colorado Springs	0	0	0	1	0	0	1	0	0	19
Denver	4	7	0	4	7	0	3	0	64	81
Pueblo	0	0	3	1	1	0	0	0	0	11
New Mexico:										
Albuquerque	0	0	1	3	0	0	0	1	1	10
Arizona:										
Phoenix	0	6	1	0	0	0	0	0	0	
Utah:										
Salt Lake City	0	0	2	1	0	0	0	0	27	39
Washington:										
Seattle	0	0	0	4	2	0	2	0	29	73
Spokane	0	0	0	0	6	0	0	0	6	36
Tacoma	0	0	0	0	0	0	0	1	15	31
Oregon:										
Portland	1	0	1	3	1	0	1	0	1	72
Salem	0	1	0	0	0	0	0	0	0	
California:										
Los Angeles	3	2	0	11	2	6	0	21	0	59
Sacramento	0	0	0	2	0	0	1	0	11	35
San Francisco	0	0	0	0	4	2	0	5	1	173

State and city	Meningitis, meningococcus		Poliomyelitis cases	State and city	Meningitis, meningococcus		Poliomyelitis cases			
	Cases	Deaths			Cases	Deaths				
Massachusetts:										
Boston	0	0	1	West Virginia:						
New York:										
Buffalo	2	0	1	Huntington	1	1	0			
New York	1	1	16	South Carolina:						
New Jersey:										
Camden	0	0	1	Charleston	0	0	1			
Newark	0	0	1	Georgia:						
Pennsylvania:										
Philadelphia	1	0	2	Atlanta	0	0	6			
Ohio:										
Cleveland	3	0	27	Savannah	0	0	4			
Toledo	0	0	1	Florida:						
Illinois:										
Chicago	0	0	6	Miami	0	0	1			
Michigan:										
Detroit	0	0	3	Tampa	0	0	1			
Flint	0	0	1	Tennessee:						
Wisconsin:										
Superior	0	0	3	Knoxville	0	0	1			
Minnesota:										
St. Paul	0	0	10	Alabama:						
Maryland:										
Baltimore	2	1	9	Birmingham	1	1	9			
District of Columbia:										
Washington	0	0	2	Montgomery	0	0	2			
Virginia:										
Norfolk	0	0	1	Louisiana:						
Texas:										
New Orleans								0	0	1
Shreveport								0	1	1
Utah:										
Salt Lake City								0	0	2
Oregon:										
Portland								0	1	0
California:										
Los Angeles								0	0	2

Encephalitis, epidemic or lethargic.—Cases: New York, 1; St. Paul, 1; Sioux City, 2; Grand Forks, 2; Minot, 9; Aberdeen, 4; Sioux Falls, 2; Baltimore, 1. Deaths: New York, 2; St. Paul, 1; Baltimore, 1.

Pellagra.—Cases: Charleston, S. C., 2; Atlanta, 1; Savannah, 2.

Typhus fever.—Cases: New York, 1; Atlanta, 1; Savannah, 4; Miami, 6; Birmingham, 1; New Orleans, 2; Fort Worth, 3; Houston, 1.

Rates (annual basis) per 100,000 population for a group of 89 selected cities (population, 1940, 33,897,000)

Period	Diph- theria cases	Influenza		Meas- les cases	Pneu- monia deaths	Scar- let fever cases	Small- pox cases	Tuber- culosis deaths	Ty- phoid fever cases	Whoop- ing cough cases
		Cases	Deaths							
Week ended Aug. 9, 1941...	6.2	3.4	1.4	50.5	34.0	23.2	0.0	50.8	5.7	197.5
Average, 1936-1940.....	10.9	3.7	1.6	55.2	40.0	35.0	4.7	52.4	10.3	207.4

FOREIGN REPORTS

CANADA

Provinces—Communicable diseases—Week ended July 19, 1941.—During the week ended July 19, 1941, cases of certain communicable diseases were reported by the Department of Pensions and National Health of Canada as follows:

Disease	Prince Edward Island	Nova Scotia	New Brunswick	Quebec	Ontario	Manitoba	Saskatchewan	Alberta	British Columbia	Total
Cerebrospinal meningitis		5		2	4			1	3	15
Chickenpox		1		35	83	7	30	23	10	189
Diphtheria	1	12	1	14	5	8	2	1		39
Dysentery				1					1	2
Influenza		9			3				30	42
Measles			3	154	176	10	12	4	38	397
Mumps				37	53	3	13	3	2	111
Pneumonia		1			3	1			2	7
Poliomyelitis						47				47
Scarlet fever		2	7	44	76	1	3	7	4	144
Smallpox								1		1
Tuberculosis	2	6	7	168	39	5		1		228
Typhoid and paratyphoid fever		1	1	21	1	1		1	1	27
Whooping cough		4		81	170		5		23	283

Poliomyelitis.—During the week ended August 22, 1941, 162 cases of poliomyelitis were reported in the Province of Manitoba, bringing the total number of cases to 597, of which 188 cases originated in Winnipeg. The mortality rate has been 2 percent. In the Province of New Brunswick for the week ended August 16, 135 cases of poliomyelitis with 9 deaths were reported and during the following week 146 cases were reported up to August 22, including 1 case in St. George, Charlotte County, near the Bay of Fundy.

Manitoba—Encephalitis.—For the week ended August 22, 1941, 127 new cases of encephalitis were reported in the Province of Manitoba making a total of 149 cases to date, with a death rate of 9 percent. Daily reports of new cases give no evidence of abatement of the epidemic.

CUBA

Habana—Communicable diseases—4 weeks ended July 26, 1941.—During the 4 weeks ended July 26, 1941, certain communicable diseases were reported in Habana, Cuba, as follows:

Disease	Cases	Deaths	Disease	Cases	Deaths
Diphtheria	17	2	Scarlet fever	1	
Leprosy	1		Tuberculosis	5	5
Malaria	6	1	Typhoid fever	45	5
Measles	19	3			

Provinces—Notifiable diseases—4 weeks ended July 19, 1941.—During the 4 weeks ended July 19, 1941, cases of certain notifiable diseases were reported in the Provinces of Cuba as follows:

Disease	Pinar del Río	Habana ¹	Matanzas	Santa Clara	Camaguey	Oriente	Total
Cancer.....	2		1	6		5	14
Chickenpox.....						6	6
Diphtheria.....		13	1	1			15
Hookworm disease.....		20		2		4	26
Leprosy.....						1	1
Malaria.....	26	8	1	15	4	238	292
Measles.....		14	4				18
Poliomyelitis.....					1		1
Scarlet fever.....						1	1
Tuberculosis.....	26	16	15	30	3	33	123
Typhoid fever.....	16	80	25	56	22	40	239
Undulant fever.....						1	1
Whooping cough.....		1	3			1	5

¹ Includes the city of Habana.

SWEDEN

Notifiable diseases—May 1941.—During the month of May 1941, cases of certain notifiable diseases were reported in Sweden as follows:

Disease	Cases	Disease	Cases
Cerebrospinal meningitis.....	19	Poliomyelitis.....	8
Diphtheria.....	3	Scarlet fever.....	1,435
Dysentery.....	39	Syphilis.....	24
Epidemic encephalitis.....	1	Typhoid fever.....	3
Gonorrhoea.....	750	Undulant fever.....	5
Paratyphoid fever.....	19		

WORLD DISTRIBUTION OF CHOLERA, PLAGUE, SMALLPOX, TYPHUS FEVER, AND YELLOW FEVER

From medical officers of the Public Health Service, American consuls, International Office of Public Health, Pan American Sanitary Bureau, health section of the League of Nations, and other sources. The reports contained in the following tables must not be considered as complete or final as regards either the list of countries included or the figures for the particular countries for which reports are given.

CHOLERA

[C indicates cases; D, deaths]

NOTE.—Since many of the figures in the following tables are from weekly reports, the accumulated totals are for approximate dates.

Place	January–June 1941	June 1941	July 1941—week ended—			
			5	12	19	26
ASIA						
China:						
Canton.....	C 131	65				
Hong Kong.....	C 832	165	62	59		
Macao.....	C 162	247		80		52
Shanghai.....	C		3	6	8	19
India:						
Calcutta.....	C 1,668	153				
Rangoon.....	C 46					
India (French).....	C 21					
Japan: Taiwan.....	C 12					

¹ For February and March.

WORLD DISTRIBUTION OF CHOLERA, PLAGUE, SMALLPOX, TYPHUS FEVER, AND YELLOW FEVER—Continued

PLAGUE

[C indicates cases; D, deaths]

Place	January-May 1941	June 1941	July 1941—week ended—			
			5	12	19	26
AFRICA						
Belgian Congo.....	C	3	3			
British East Africa:						
Kenya.....	C	26	42			
Uganda.....	C	58	6			
Egypt: Port Said.....	C			8		
Madagascar.....	C	191	3			
Morocco.....	C	1,144	344	90	63	61
Casablanca. ¹	C					60
Tunisia: Tunis.....	C	2				
Union of South Africa.....	C	59				
ASIA						
China: Foochow.....	C	3				
Dutch East Indies:						
Java and Madura.....	C	301				
West Java.....	C	205				
India:						
Calcutta.....	C	3				
Rangoon.....	C	6				
Indochina (French).....	C		17			
Palestine: Haifa.....	C					2
Plague-infected rats.....		10				
Thailand: Lampang Province.....	C	1				
NORTH AMERICA						
Canada—Alberta—Plague-infected ground squirrel.....			1			
SOUTH AMERICA						
Argentina:						
Cordoba Province.....	C	116	5			
Santa Fe Province—Plague-infected rats.....		67				
Peru:						
Ancash Department.....	C	1				
Lambayeque Department.....	C	2				
Libertad Department.....	C	6				
Lima Department.....	C	6				
Moquegua Department—Ilo.....	C	4	3			
Piura Department.....	C	2				
OCEANIA						
Hawaii Territory: ⁴ Plague-infected rats.....		35	9	3		
New Caledonia.....	C	9				

¹ A report dated June 23, 1941, stated that an outbreak of plague had occurred in Casablanca, Morocco where several deaths had been reported.

² Includes 2 cases of pneumonic plague.

³ Includes 1 case of pneumonic plague.

⁴ During April and May, 4 lots of plague-infected fleas were reported in Hawaii Territory.

SMALLPOX

[C indicates cases; D, deaths]

AFRICA						
Algeria.....	C	122	24	18		
Belgian Congo.....	C	48				
British East Africa.....	C	17	2			
Dahomey.....	C	452	2	6	4	
French Guinea.....	C	45				
Ivory Coast.....	C	30	2	7		
Morocco.....	C	31				
Nigeria.....	C	607	30			
Niger Territory.....	C	221	8	9	11	9
Portuguese East Africa.....	C	9				
Rhodesia: Southern.....	C	86				
Senegal.....	C	52	4	1		2
Sierra Leone.....	C	15				
Sudan (Anglo-Egyptian).....	C	7				
Sudan (French).....	C	19				
Union of South Africa.....	C	94				

WORLD DISTRIBUTION OF CHOLERA, PLAGUE, SMALLPOX, TYPHUS FEVER, AND YELLOW FEVER—Continued

SMALLPOX—Continued

[C indicates cases; D, deaths]

Place	January— May 1941	June 1941	July 1941—week ended—			
			5	12	19	26
ASIA						
Ceylon.....	C 40	12				
China.....	198	18	4	3	2	1
Chosen.....	464					
India.....	10,580	35				
India (French).....	6					
India (Portuguese).....	44					
Indochina (French).....	702	120		37		26
Iran.....	8					
Iraq.....	919					
Japan.....	127					
Straits Settlements.....	1					
Syria.....	1					
Thailand.....	218	13				
EUROPE						
France.....	C 1					
Portugal.....	C 26	5				
Spain.....	C 129					
NORTH AMERICA						
Canada.....	C 22					
Dominican Republic.....	C 2					
Guatemala.....	C 5					
Mexico.....	C 22					
SOUTH AMERICA						
Brazil.....	C 1	1				
Colombia.....	C 281	2				
Paraguay.....	C 8					
Peru.....	C 249					
Uruguay.....	C 7					
Venezuela (alastrim).....	C 154	7		1		

TYPHUS FEVER

[C indicates cases; D, deaths]

AFRICA						
Algeria.....	C 5,597	1,964		578		
British East Africa: Kenya.....	C 12					
Egypt.....	C 4,214					
Morocco.....	C 385	252	39	39	23	32
Sierra Leone.....	C 5					
Tunisia.....	C 2,764	962	191	183	79	
Union of South Africa.....	C 116	2				
ASIA						
China.....	C 152	25				
Chosen.....	68					
Iran.....	105					
Iraq.....	37					
Japan.....	295	2				
Palestine.....	23					
Straits Settlements.....	C 4	1				
EUROPE						
Bulgaria.....	C 145	34	7	2		3
Germany.....	C 824	191	28	33	67	
Gibraltar.....	C 2					
Greece.....	C 7					
Hungary.....	C 233	60	2			
Irish Free State.....	C 26					
Poland.....	C 530					
Portugal.....	C 5					
Rumania.....	C 562	16	4	12		2
Spain.....	C 2,693	1,674				
Switzerland.....	C 2	3				
Turkey.....	C 543					
Yugoslavia.....	C 78					

1 For the month of April.

**WORLD DISTRIBUTION OF CHOLERA, PLAGUE, SMALLPOX, TYPHUS
FEVER, AND YELLOW FEVER—Continued**
TYPHUS FEVER—Continued

[C indicates cases; D, deaths]

Place	January- May 1941	June 1941	July 1941—week ended—			
			5	12	19	26
NORTH AMERICA						
Guatemala.....	C 103	6				
Mexico.....	C 61	2			1	
Panama Canal Zone.....	C 3					
SOUTH AMERICA						
Bolivia.....	C 75					
Brazil.....	C	1				
Chile.....	C 66	9	1			
Ecuador.....	C 65					
Peru.....	C 453					
Venezuela.....	C 26	5				
OCEANIA						
Australia.....	C 8					
Hawaii Territory.....	C 13	3	1			

YELLOW FEVER

[C indicates cases; D, deaths]

AFRICA						
Belgian Congo:						
Kimvulu.....	C		1			
Libenge.....	C		1			
French Equatorial Africa:						
Gabon.....	C 2					
Mayumba.....	C		4			
Gold Coast: Accra.....	C 1					
Ivory Coast ¹	C 3			1		
Spanish Guinea.....	D 4					
SOUTH AMERICA⁴						
Brazil:						
Bahia State.....	D		2			
Para State.....	D		1			
Colombia:						
Antioquia Department.....	D 2					
Boyaca Department.....	D 6		1		1	
Intendencia of Meta.....	D 2		2			
Santander Department.....	D 3		1			
Tolima Department.....	D 1					
Peru: Junin Department.....	C 5					

¹ During the week ended Aug. 9, 1 fatal case of yellow fever was reported in Dimbokro, Ivory Coast.² Includes 2 suspected cases.³ Suspected.⁴ All yellow fever reported in South America is of the jungle type unless otherwise specified.

X