PUBLIC HEALTH REPORTS

VOL. 49 MARCH 23, 1934 NO. 12

VIABILITY OF ENDAMOEBA HISTOLYTICA AND ENDAMOEBA COLI

Effect of Drying

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In connection with studies of the sources of infections of amoebic dysentery, it appeared desirable to determine the time during which the causative organism remained viable when smeared on the hands. This became especially important, since many students of amoebiasis have considered that the infection was spread largely through direct transfer on food of *Endamoeba*-cyst-bearing fecal material from carriers to well persons, in whom infection was thus established. It is generally accepted by students of protozoa that living forms of intestinal protozoa, especially *Amoebae,* may be distinguished from dead forms by staining the preparation with eosin. If the parasite takes up the stain from a solution (aqueous) of 1:1000 eosin, the organism is considered dead; while if it refuses to take the stain, it is to be regarded as alive.

To those not familiar with the test it is rather surprising to note the sharp differentiation to be effected by the procedure. The method employed in these experiments was as follows, with such variations as are noted under individual tests.

The fingers and thumb in some instances were dosed with a 24-hour culture of *Escherichia coli,* in order that the effect of drying on this organism might be contrasted with the effect of drying on *Endamoeba histolytica.* The stool specimen containing a sufficient number of the cyst forms of *Endamoeba histolytica* was smeared on the fingers of the healthy volunteer, or the fingers were dipped into a homogenous stool emulsion. In either case, the material was allowed to dry.

The amount of fecal material put on the fingers was quite liberal. At varying periods of time after the contamination of the fingers or thumb, the fingers and thumb were immersed in a sterile centrifuge tube containing sterile distilled water or normal salt solution, and the feces washed off as completely as practicable.

The suspension was rotated at low speed in the centrifuge for about 5 minutes. At the end of this time a drop of the sediment was collected with a pipette and mixed on a glass slide with an approximately equal volume of the eosin solution. The mixture was then examined with dry objectives, using first the low power, and, when necessary for complete identification, the higher power.

In experiments where counts were made, all *Amoebae* on the microscopic preparation were enumerated. Throughout the experiment the temperature of the laboratory was at 27° to 29°C.

In the absence of any information on the longevity of *Endamoeba histolytica* cysts on human hands, a number of preliminary experiments were necessary to determine the starting point of washing the individual fingers after contaminating them.

PRELIMINARY EXPERIMENTS

Materials.—Twenty-four-hour-old stool containing many *Endamoeba histolytica* cysts, kept in ice-box.

Twenty-four-hour culture of *Esch. coli*, Endo plates, sterile distilled water, sterile 50-cc centrifuge tubes.

The fingers and thumbs of both hands were used.

Procedure.—The fingers and thumbs of both hands were first contaminated with the 24-hour culture of *Esch. coli.* They were dipped immediately in a beaker containing an even emulsion of the stool (the stool was well emulsified in sterile distilled water and strained through one layer of gauze into a sterile beaker).

The hands were then held over a small, clean basin to collect any drippings. After the intervals shown in the tables, a finger or thumb was washed in a large sterile centrifuge tube about two thirds full of sterile distilled water.

When the experiment was completed, these washings were centrifuged at low speed for about 4 minutes. The sediment was cultured for *Esch. coli* and examined microscopically for *E. histolytica* cysts, using 1:1000 aqueous solution of eosin.

Results.—The following tables present the results of the preliminary experiments:

EXPERIMENT 1

Date	Material used	Period of drying—Interval between contamination and washing of fingers			
		50 min.	80 min.	110 min.	140 min.
Feb. 28, 1934	Esch. coli	Dead	Dead	Dead	Dead.
	E. histolytica cysts	do	do	do	Do.

EXPERIMENT 2

Date	Material used	Period of drying—Interval between contamination and washing of fingers			
		10 min.	15 min.	20 min.	25 min.
Mar. 1, 1934	Esch. coli	Dead	Dead	Dead	Dead.
	E. histolytica cysts	do	do	do	Do.

EXPERIMENT 3

		3 min.	4½ min.	5½ min.	6½–7½ min.
Mar. 1, 1934	Esch. coli	Viable	Viable	Viable	Viable.
	E. histolytica cysts	do	Few viable; mostly dead.	Dead	Dead.

EXPERIMENT 4

		3 min.	4 min.	5 min.	6 min.
Mar. 3, 1934	Esch. coli	Viable	Viable	Viable	
	E. histolytica cysts	do	Few viable; mostly dead.	Dead	

Conclusion.—This series of experiments shows that this strain of *Endamoeba histolytica* cysts dies within 5 minutes on human hands when spread in the concentration indicated above and allowed to dry at room temperature, and that *Esch. coli* is more resistant to drying than the strain of *Endamoeba histolytica* cysts used.

LATER EXPERIMENTS

In classifying *Endamoeba histolytica* cysts as "large" and "small", as found in certain of the reports of these experiments, an opinion was based on the general impression given the observer, but without the making of measurements with a micrometer. There is a difference of opinion among protozoologists as to whether the size of the cysts is a matter of any significance.

Experiment 5 (Mar. 5, 1934).—The stool specimen was about 5 hours old and came from a clinically active case of dysentery of several weeks' duration. The specimen was semiliquid and brownish in color. No blood was evident to naked-eye examination. Direct microscopic examination showed some motile forms (trophozoites) and a moderate number of cysts, both large and small varieties being represented, the latter more numerous.

After preliminary dosing of the hands with a culture of *Esch. coli*, the fecal specimen was applied liberally enough to leave a very distinct brownish film. The film was dry after about 3 minutes. The results of examinations made at various intervals after the smearing (not after drying) are shown here.

After 5 minutes: About half the cysts living and half dead.
After 7 minutes: The dead cysts far outnumber the living.
After 9 minutes: All cysts dead.
After 10 minutes: One cyst living; 10 dead.
After 11 minutes: One small cyst living; several small cysts dead. All large cysts dead.

A control specimen of the material used but not dried showed at the end of the experiment 13 small cysts living, none dead. Of large cysts counted, 5 were living and 1 was dead.

It is to be expected that in any preparation a certain number of protozoa will be dead in the natural course of events without reference to experimental conditions.

At this stage of the work it seemed desirable to ascertain the resistance of cysts of *E. coli.*

Experiment 6 (Mar. 5, 1934).—A formed stool specimen from a healthy 20-year-old female was available, which specimen contained *E. coli* in cyst form only. The specimen was about 6 hours old when used.

The results were in striking contrast with those obtained with *E. histolytica,* since at the end of 15 minutes none of the cysts was found dead. Accordingly, a modification of the test was run by drying an emulsion of the same stool specimen, 30 hours old, on a glass slide.

Experiment 7 (Mar. 5, 1934)—E. coli cysts.[1]—After varying periods, as shown in the accompanying tabulation, the dried films were restored to the form of an emulsion, the eosin solution was added, and the cysts were counted, with the results shown in the table.

In this test and in all other tests where counts are shown all of the protozoa in the usable field were counted. The results are as follows:

	Living	Dead		Living	Dead
After 5 minutes' drying	7	0	After 40 minutes' drying	30	1
After 10 minutes' drying	11	0	After 60 minutes' drying	30	3
After 15 minutes' drying	13	0	After 90 minutes' drying	26	3
After 20 minutes' drying	25	0	After 120 minutes' drying	33	1
After 30 minutes' drying	30	2			

Experiment 8 (Mar. 6, 1934).—This test was carried out to determine the effect of drying the cyst-bearing fecal emulsion of *Endamoeba histolytica* on glass slides.

A brown semisolid stool from a clinical case of dysentery was available. The specimen had been kept in the icebox for 28 hours. An emulsion was made in distilled water. The specimen contained both large and small cysts of *Endamoeba histolytica,* the small variety predominating.

[1] Since no count for living and dead cysts was made on the fecal specimens used in the test, the results of the test are not to be interpreted as showing a mortality from drying, as the number shown as dead in the tabulation may have been dead in the original specimen.

One drop of the emulsion was placed toward each end of the glass slide. The drop on one end was spread out with a wooden applicator stick to permit drying, while that at the other end served as a control, not being spread.

It was found, as shown in the following table, that the emulsion did not dry nearly so quickly as on the fingers; only after about 10 minutes was the spread quite dry. The counts were as follows:

	Test (spread)				Control (not spread)			
	Small cyst		Large cyst		Small cyst		Large cyst	
	Living	Dead	Living	Dead	Living	Dead	Living	Dead
2 minutes after spreading	7	1	2		10		3	
5 minutes after spreading	9		2		8			
7 minutes after spreading	11	2	1	2	9	1	2	
10 minutes after spreading	2	4	1	3	10		5	
15 minutes after spreading	3	14		5	12		1	3

Experiment 9 (Mar. 6, 1934).—This experiment was carried out in the same manner as was the preceding one, save that small drops were spread out on a larger surface with the object of promoting rapid drying. The results are shown below:

	Test (spread)				Control (not spread)			
	Small cyst		Large cyst		Small cyst		Large cyst	
	Living	Dead	Living	Dead	Living	Dead	Living	Dead
2 minutes after spreading	6	4	4		14	4	1	1
5 minutes after spreading	2	11	1	6	15	1	4	
7 minutes after spreading	4	11	1	4	16	1	3	
10 minutes after spreading	3	11		6	17		2	1
15 minutes after spreading	2	10		8	17		3	

Experiment 10 (Mar. 6, 1934).—In the next experiment a comparison was made between the times of drying required to kill when the material, a fecal emulsion 9 days old containing the large variety of cysts, was dried on the hand and on a dry rubber glove on the hand. The counts are shown in the following tabulation:[2]

	On the hand		On rubber glove on the hand	
	Living	Dead	Living	Dead
3 minutes after spreading	5	4	5	
5 minutes after spreading		2	1	5
7 minutes after spreading		8	1	8
10 minutes after spreading		12		4
15 minutes after spreading		9		9

[2] It was noted that the emulsion on the hand dried before that on the rubber glove.

Experiment 11 (*Mar. 7, 1934*).—In this experiment a fresh stool specimen one half hour old, containing blood and mucus, with many motile forms (trophozoites), from an active clinical case of dysentery was employed. The individual had had symptoms of dysentery for about 2 months.

The object here was to determine whether, under the conditions of the experiment, the motile forms (trophozoites) were as readily destroyed by drying as had been believed. The specimen on the fingers dried in about 3 minutes, the mucous flakes remaining moist rather longer than the remainder of the preparation.

The preparations were made after complete drying and at the intervals shown in the following tabulation: [3]

	Live	Dead
1 minute after drying	24	9
2 minutes after drying	None	Many
3 minutes after drying	None	Many
5 minutes after drying	None	Many
10 minutes after drying	None	Many

Experiment 12 (*Mar. 7, 1934*).—There was available for this experiment a 40-hour-old culture of *Endamoeba histolytica* growing on Williamson's liver infusion agar, overlaid with a sterile mixture of Wassermann-negative human serum and saline in the proportion 1:6. The culture had been transferred every 48 hours for several months. The cultures contained many motile forms (trophozoites), a few precystic forms, and a very few cysts.

The number of organisms found in the saline suspension after the culture had been dried on the hand was so small that counts were unsatisfactory, though all to be found in each preparation were enumerated. The following table gives the counts:

	Living	Dead		Living	Dead
1 minute after spreading	m 4	3	5 minutes after spreading	m	4
	p 1	------		p	------
	c	------		c	------
2 minutes after spreading	m 1	1	10 minutes after spreading	m	6
	p 4	1		p	------
	c	1		c	------
3 minutes after spreading	m	4	Control	m 6	------
	p 1	------		p 15	------
	c	1		c	------

m=Motile forms. p=Precyst forms. c=Cysts.

Experiment 13 (*Mar. 8, 1934*).—The stool available was about 6 hours old, and came from a clinically active case of dysentery. It contained a moderate number of cyst forms, both large and small,

[3] In all tests in which motile forms (trophozoites) were used, the suspensions were made in 0.85 saline, as distilled water in the preparations was found unsuitable.

and a few in the precyst stage. The patient had had recurrences of symptoms of amoebic dysentery for 8 months, and had been given treatment.

The results of the count at the end of the experiment, in which the fecal matter was spread on the hands, are as follows:

	Large		Small	
	Living	Dead	Living	Dead
3 minutes after spreading	11	1	9	0
5 minutes after spreading	----------	9	3	4
7 minutes after spreading	----------	4	1	4
10 minutes after spreading	----------	4	----------	2
Control (kept moist)	20	1	14	4

Experiment 14 (*Mar. 9, 1934*).—A 4-hour-old soft-stool specimen was available for this test. It came from a case with mild clinical symptoms of amoebic dysentery that had been treated with amoebicides. No blood was visible, but there was some mucus, and on microscopic examination many cysts of *E. histolytica* of the small variety were seen.

The undiluted specimen was used for smearing the fingers and thumb. The results are shown in the following table:

	Live	Dead		Live	Dead
2 minutes after smearing	26	23	8 minutes after smearing	3	115
4 minutes after smearing	5	10	10 minutes after smearing	1	20
6 minutes after smearing	3	55	Control (undried material)	68	3

This test was varied, using the same material in the same manner but with the specimen 7 hours old and with a change in time intervals after smearing. The results were as follows:

	Live	Dead
2 minutes after smearing	143	54
3 minutes after smearing	11	59
4 minutes after smearing	6	65
5 minutes after smearing	1	107
7 minutes after smearing	----------	34
Control	112	13

COMMENT

The conditions of the experiments provided for a fouling of the hands far in excess of any that would be likely to occur under ordinary conditions, even with the most untidy or willfully careless carrier. Nevertheless, the number of cysts of *Endamoeba histolytica* to survive beyond 5 minutes was very small in proportion to those killed, and it was exceptional that any survived beyond 10 minutes.

THE AMERICAN DOG TICK, DERMACENTOR VARIABILIS, AS A HOST OF BACTERIUM TULARENSE [1]

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In 1924, Parker, Spencer, and Francis reported the recovery of *Bacterium tularense* from Rocky Mountain wood ticks, *Dermacentor andersoni*, in nature, and from experimentally infected rabbit ticks, *Haemaphysalis leporis-palustris*. They also showed experimental transmission by the various stages of the former species. Since that time, experimental data and accumulating information of human infection have shown the importance of tick-borne tularaemia. Natural infection in ticks of the following species has been reported: *H. leporis-palustris* (Parker and Spencer, 1927); the Pacific coast tick, *D. occidentalis* (Parker, Brooks, and Marsh, 1929); the American dog tick, or sometimes called the eastern wood tick, *D. variabilis* (Green, 1931); and the bird tick, *H. cinnabarina* (Parker, Philip, and Davis, 1932).

The wide distribution of *D. variabilis* within that part of North America in which tularaemia occurs naturally and its record as a human pest make this species of potential importance as a vector of the disease over a considerable area. Hanson and Green (1929) have reported a case associated with tick bite in Hubbard County, Minnesota. Belote (1931), reporting a case in Michigan with primary ulcer on the abdominal wall, states: "Wood ticks cannot be ruled out as a possible source of the infection." *D. variabilis* is the tick of importance as a parasite of man in both these States. A number of "tick-bite" cases have also occurred in the southeastern and south central States, but the species of tick or ticks concerned are uncertain. Francis (1927) stated that "Tick-bite has caused 17 cases in Arkansas, Oklahoma, Texas, Louisiana, and Tennessee", and he now states that by 1933, tick-bite cases in southern States have increased from 17 to 58, and the States named have been increased by the addition of Virginia, North Carolina, Georgia, Missouri, Kansas, and Illinois. Kerlin (1929) reported 3, and possibly 4, cases due to tick bite in Louisiana. *D. variabilis* and *Amblyomma americanum* (the lone star tick) are the species of ticks commonly attacking man in most parts of the region concerned.

In consideration of the above facts, tests of transmission of *Bact. tularense* were undertaken with both species of ticks. Each has proved to be an efficient experimental vector. Only the results of tests with *D. variabilis* are reported in this paper, however.

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[1] Contribution from the Rocky Mountain Spotted Fever Laboratory of the United States Public Health Service at Hamilton, Mont.

EXPERIMENTATION

Lots 10901 to 10904.—In May 1929 adult *D. variabilis* were received from Dr. W. A. Riley, of the University of Minnesota. These were fed on tularaemia-infected rabbits. Results of test feedings of progeny of these adults on guinea pigs and rabbits were negative or inconclusive, and the lots were discontinued.

Lot 12901.—On June 29, 1931, rabbit 6744 was infested with larvae from engorged adults forwarded by Dr. C. M. Pierce of Chadron, Nebr., in May. On the next day, the animal was inoculated dermally from the spleen of an infected guinea pig. July 6 (one week after infestation), the engorged larvae were recovered and the rabbit was sacrificed. Typical gross lesions of tularaemia were noted in the spleen and liver.

On July 29 normal guinea pig 34020 was infested with nymphs reared from the above larvae. Seven days later 22 engorged nymphs were recovered. On the ninth day after infestation the host animal was found dead and 4 more engorged nymphs were obtained. Typical gross lesions of tularaemia were observed at necropsy. Guinea pig 34021 was infested with another group of nymphs from the above-mentioned larvae and a total of 49 became engorged. This animal died on the eighth day and revealed characteristic lesions of the disease.

On August 5, one engorged nymph that fed on the former of the above-mentioned guinea pigs and 2 that fed on the latter pig were inoculated separately into guinea pigs 34115, 34116, and 34117. Two of these died on the fourth day and the other on the sixth day, all showing typical gross lesions of tularaemia at necropsy.

On October 15, 20, and 26, adult *D. variabilis* reared at room temperature from the nymphs of the above 2 test feedings, were placed on guinea pigs 35514 and 35515, respectively. Twelve and fifteen days later the ticks were removed in a poorly fed condition and stored at room temperature. The first guinea pig died on November 11, 26 days after infestation. No evidence of tularaemia was observed at necropsy. Owing to considerable post mortem change, the cause of death was uncertain and no transfer was made. The other test animal (35515) died of pneumonia on November 3, 18 days after infestation, with no gross evidence of tularaemia. However, spleen tissue transferred subcutaneously to a normal guinea pig caused death on the third day, the necropsy findings being typical.

On October 31, 5 of the poorly fed adults from each of guinea pigs 35514 and 35515, referred to above, were eviscerated and their tissues injected into guinea pigs 35517 and 35598, respectively. No. 35517 showed no reaction and was killed on the eleventh day, revealing no evidence of tularaemia at necropsy. No. 35598 died in 3 days of pneumonia. Spleen transfer, however, to guinea pig 35765 resulted in typical infection fatal in 3 days, and a pure culture of *Bact. tularense* was obtained from heart blood taken just before death.

The remainder of these adult ticks were placed on guinea pigs 36204 and 36205 on November 30. No perceptible reaction had occurred by January 6, 1932, and the animals were discarded. The tests of these lots were discontinued because of poor feeding.

Lot 14301.—The original stock for this lot consisted of unfed adults received from Dr. R. G. Green, Lake Alexander, Minn., in June 1932. Guinea pig 44001 was infested with 13 males and 21 females on September 6, 1932, This animal was then inoculated dermally on September 10, and died 4 days later with typical gross lesions. A total of 26 engorged females were recovered and placed over damp sand at room temperature for oviposition.

Two normal guinea pigs, 44931 and 44933, were each injected intraperitoneally with about 100 eggs from 2 different females of this lot without producing any apparent reaction over an observation period of 25 days. However, inoculation of the viscera of 3 partly fed adults of the same lot was fatal in 5 days to guinea pig 44129. Necropsy findings were typical, proving that opportunity of ingesting *Bact. tularense* had been provided the adults of this lot.

Groups of larvae reared from several of the above-mentioned female ticks were infested on 6 guinea pigs, 1 domestic rabbit, and 1 native white-footed mouse (*Peromyscus maniculatus artemisiae*) on November 21 and December 2. The mouse died in 3 days without evident lesions, and no transfer was attempted. (Death may have been due to tularaemia. See duplicate test, lot 14302.) The rabbit was bled and killed on the thirteenth day. No suggestive lesions were observed at necropsy, and a negative agglutination test for *Bact. tularense* was obtained with the blood. The guinea pigs showed no reactions during periods of 19 to 47 days and, when killed, revealed no evidence of tularaemia.

Tests of this lot were discontinued.

Lot 14302.—Original stock of *D. variabilis* adults were from the same source as the preceding. Conditions of infection, using guinea pig 44002, were exactly the same. Fifteen male and 21 female ticks were applied on September 6. Dermal inoculation of the host was made 4 days later, death resulting in another 4 days. Sixteen fully engorged females were recovered during the 2 days preceding the death of the host animal, and were segregated for subsequent testing. The guinea pig showed typical gross lesions at necropsy.

Each of two normal guinea pigs, 44932 and 45459, were injected with approximately 100 eggs, each group of eggs being from a different female tick recovered at death of guinea pig 44002. Periods of 25 and 45 days elapsed without observed reaction, and when the animals were killed no evidence of tularaemia was discerned.

Seven guinea pigs, 2 rabbits, and 1 white-footed mouse were infested with different groups of larvae from several of the above-mentioned female ticks. The guinea pig tests were all negative, as in the preceding experiment, and the injection of pooled engorged larvae from these animals was likewise without result. The rabbit tests were also negative, both by agglutination (blood drawn on the thirteenth and twenty-fifth days) and at necropsy when killed. On the other hand, positive results in guinea pigs followed tissue transfer in series from the mouse, which died without evident lesions on the fifth day after infestation. Transfer by spleen of this mouse resulted in acute peritonitis, fatal within 24 hours; that by lung tissue caused death of another animal on the second day, again without definite lesions. Transfer from the latter animal, dermally by spleen and subdermally by spleen and liver, was made to three guinea pigs. These died on the fourth and fifth days, and necropsy revealed typical lesions. Pure cultures of *Bact. tularense* were isolated from heart blood drawn while the animals were moribund.

Sixteen partially fed larvae from the above mouse were macerated and inoculated into 2 additional guinea pigs. These animals were moribund 8 days later, and at that time pure cultures of *Bact. tularense* were obtained from heart blood. Both died the next day and typical gross lesions were noted. Dermal transfers by spleen to two other animals caused typical infections, fatal in 5 days.

Fifteen nymphs reared from 1 of the 2 above-mentioned rabbits were placed on guinea pig 46465 on December 31. The animal died the eighth day without lesions, and intraperitoneal transfer by spleen injection in series to 2 additional guinea pigs resulted in peritonitis in the second animal. However, 2 partly engorged nymphs inoculated into guinea pig 46792 caused typical infection fatal on the third day. A pure culture of *Bact. tularense* was obtained from heart blood drawn just prior to death of the animal.

Ten, six, and one nymphs from larvae fed on the white-footed mouse were fed on normal guinea pigs 46466, 46692, and 48447, respectively. The first died in 5 days without evidence of tularaemia, and a culture of heart blood when moribund was negative. No. 46692 died of pneumonia on the twelfth day without evidence suggestive of tularaemia in either the spleen or liver. Subcutaneous injection of the spleen into another animal was negative, as was also an agglutination test of the heart blood drawn on the twenty-sixth day. The lone nymph was dead on the third guinea pig *in situ* on the fourth day, and the animal died on the ninth day without evident cause of death. Spleen transfer, intraperitoneally, was without result, and heart blood of the twenty-third day contained no specific agglutinins. However, intraperitoneal inoculation of an emulsion of 5 partly fed nymphs from 46692 (4 of which were dead and the other dying *in situ*) caused the death of guinea pigs 47246 and 47247 in 5 days; necropsy findings were characteristic, and heart blood of each, drawn just prior to death, yielded pure cultures of *Bact. tularense.* Transfer by spleen dermally from one of the above-mentioned animals also produced typical infection, fatal on the fifth day.

Lot 14305.—To confirm "hereditary transmission", a group of adults received from Ono, Calif., on June 14, 1933, as partially engorged females from a dog, were placed on guinea pig 52625 two days after dermal inoculation. This animal died on the fifth day (3 days after infestation), and 4 nearly engorged females were segregated for oviposition. On August 3, about 100 eggs from each of 2 ticks were washed thoroughly in distilled water and injected intraperitoneally into separate normal guinea pigs, 53544 and 53545. These test animals died in 4 and 3 days, respectively, showing characteristic gross lesions of tularaemia at necropsy. Heart blood of the first yielded a pure culture of *Bact. tularense* and a spleen transfer dermally to a second animal was fatal in 6 days, typical gross lesions being present in both animals.

COMMENT

It is seen that stage to stage and generation to generation transmission of *Bact. tularense* in *D. variabilis* can be demonstrated experimentally, but may not be constant.

In one series of tests, hereditary continuity of infection was shown only in those larvae fed on a mouse, although two rabbits and several guinea pigs were exposed to the bites of the same larval lots. However, further evidence of hereditary transfer was supplied by positive results following the injection of separate guinea pigs with washed eggs of two infected ticks.

It is also seen that some of the nymphs of lot 14302 and adults of lot 12901 proved to contain *Bact. tularense* by later injection, did not transmit infection while feeding (for a period as long as 10 days in in the case of animal 46692, lot 14302), part of the infected ticks dying while only partially engorged and still attached to the host.

The death of ticks engorging or engorged on tularaemia-infected hosts has not infrequently been observed with *D. variabilis*, especially among ovulating females which had not detached until death of the donor guinea pig of tularaemia or among the progeny of such females. This may have some connection with the fact that in other tularaemia studies made at this laboratory it has been noted that bacteraemia in infected guinea pigs is most intense just prior to death. Because of this unusual mortality, continuous lines of tularaemia-infected ticks have frequently been difficult to maintain. The most successful procedure has been to remove attached ticks before the death of the host and to replace them on a normal animal whenever further engorgement is necessary.

This apparent deleterious effect of *Bact. tularense*, as well as the failure of some infected ticks to transfer infection while feeding, has been encountered also in tests of tularaemia transmission with other species of ticks. No comparable loss has been encountered in non-infected experimental ticks stored and fed under similar conditions.

The recovery of the bacterium from *H. cinnabarina* dead *in situ* on a recently dead sage hen in nature was reported by Parker, Philip, and Davis (1932).

The observations noted above suggest that *Bact. tularense* is not completely adapted to continued residence through successive stages of its host ticks. Nevertheless, the role of ticks in the dissemination of the disease among susceptible animals and to man is well established.

The importance and distribution of *D. variabilis* as a parasite of man is discussed elsewhere by Parker, Philip, and Jellison, 1933. While in areas where this tick is indigenous, the most frequent avenue of human infection with tularaemia is direct contact with infected animals, particularly rabbits, yet the possibility of infection by *D. variabilis* must be kept in mind, particularly if the case history fails to give evidence of animal handling.

SUMMARY

The American dog tick, *D. variabilis,* was experimentally infected with *Bact. tularense* in the adult stage and in the larval stage. Larvae from the above adults fatally infected a white-footed mouse. Resultant engorging nymphs were shown to contain virulent organisms, which, in some instances, apparently caused the death of the ticks *in situ;* however, demonstrable infection was not produced in some of the host animals. Further evidence of generation to generation continuity of *Bact. tularense* in this tick was secured by the injection of partial batches of eggs from two additional infected ticks.

Nymphs reared from infected larvae produced fatal infections in two guinea pigs. Infection was produced by resultant adults in separate guinea pigs both by feeding and by injection.

Tests with this and other species of ticks (to be reported) suggest that *Bact. tularense* is not entirely adapted to continued residence in ticks through their developmental cycle, since the ticks themselves

sometimes die (apparently as a result of the presence of this organism) while still attached to the host animal and occasionally without infecting such host.

Since (1) larval-to-adult and adult-to-progeny continuity of infection has been demonstrated, (2) recovery of infected ticks in nature has been reported, and (3) cases of human infection apparently associated with bites of this species have occurred, *D. variabilis* must be kept in mind as a possible source of human infection, especially where case histories fail to show evidence of animal contacts.

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MOST PROBABLE NUMBERS FOR EVALUATION OF COLI-AEROGENES TESTS BY FERMENTATION TUBE METHOD

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In a previous publication (1) a procedure was presented for computation of the most probable number of *coli-aerogenes* organisms from results of the fermentation tube method of bacteriological analysis of water. Employing this procedure has expedited the computation of most probable numbers corresponding to analytical results possible to be obtained from a wide variety of combinations of portions of sample planted at various dilutions. Such computed values are presented in the accompanying tabulations for the combinations of tubes most likely to be employed in routine water, sewage, and milk analyses. All values are given in organisms per hundred cubic centimeters of sample and are correct to two significant figures.

Most probable number (M.P.N.) values in heavy face type are those corresponding to the analytical results in which no "skips" or apparent inconsistencies occur. Tables 1-A and 1-B comprise values for the results of various combinations of tubes to a total of seven tubes in as many as three dilutions. Table 2 contains the most probable numbers for combinations in which 3, 4, or 5 tubes are planted in each of three dilutions in geometric series, while tables 3-A, 3-B, and 3-C are the most probable number values corresponding to all possible combinations of a total of five tubes when planted in not more than three dilutions in the series 10—1—0.1 cc, 50—10—1 cc, and 100—50—10 cc, respectively.

Table 1-A.—*Most probable numbers per 100 cc of sample, planting various portions in not more than 3 dilutions*

Number of positive tubes in dilutions	Combinations of portions planted in cubic centimeters												
Low Mid High	1-10 1-1 1-0.1	1-10 5-1 1-0.1	2-10 1-1	2-10 1-1 1-0.1	2-10 2-1 2-0.1	1-50 1-10 1-1	1-50 5-10 1-1	2-50 1-10 1-1	2-50 2-10 2-1	1-100 1-50 1-10	1-100 5-50 1-10	2-100 1-50 1-10	2-100 2-50 2-10
0 0 1	9.0	6.7	------	4.7	4.5	1.7	1.0	0.90	0.83	0.65	0.28	0.39	0.32
0 0 2	------	------	------	------	9.0	------	------	------	1.7	------	------	------	.64
0 1 0	9.4	6.8	4.9	4.9	4.6	1.8	1.0	.94	.86	.75	.30	.43	.34
0 1 1	19	14	------	9.7	9.2	3.6	2.1	1.9	1.7	1.6	.61	.88	.69
0 1 2	------	------	------	------	14	------	------	------	2.6	------	------	------	1.1
0 2 0	------	14	------	------	9.4	------	2.2	------	1.8	------	.65	------	.75
0 2 1	------	21	------	------	14	------	3.3	------	2.7	------	1.0	------	1.2
0 2 2	------	------	------	------	19	------	------	------	3.6	------	------	------	1.6
0 3 0	------	22	------	------	------	------	3.5	------	------	------	1.1	------	------
0 3 1	------	30	------	------	------	------	4.7	------	------	------	1.5	------	------
0 4 0	------	31	------	------	------	------	5.0	------	------	------	1.6	------	------
0 4 1	------	39	------	------	------	------	6.4	------	------	------	2.1	------	------
0 5 0	------	40	------	------	------	------	6.8	------	------	------	2.4	------	------
0 5 1	------	49	------	------	------	------	8.3	------	------	------	3.0	------	------
1 0 0	23	11	6.5	6.4	6.0	3.4	1.4	1.3	1.1	.98	.33	.49	.37
1 0 1	95	24	------	13	12	9.9	2.9	2.5	2.2	2.3	.67	1.0	.77
1 0 2	------	------	------	------	19	------	------	------	3.4	------	------	------	1.2
1 1 0	240	36	14	14	13	24	3.1	2.7	2.3	4.0	.72	1.2	.85
1 1 1	------	45	------	22	20	------	4.9	4.2	3.6	------	1.1	1.9	1.3
1 1 2	------	------	------	------	28	------	------	------	4.9	------	------	------	1.8
1 2 0	------	51	------	------	21	------	5.5	------	3.8	------	1.2	------	1.5
1 2 1	------	76	------	------	29	------	7.9	------	5.3	------	1.7	------	2.1
1 2 2	------	------	------	------	37	------	------	------	6.9	------	------	------	2.8
1 3 0	------	89	------	------	------	------	9.0	------	------	------	1.9	------	------
1 3 1	------	120	------	------	------	------	12	------	------	------	2.6	------	------
1 4 0	------	150	------	------	------	------	15	------	------	------	3.1	------	------
1 4 1	------	210	------	------	------	------	21	------	------	------	4.1	------	------
1 5 0	------	390	------	------	------	------	39	------	------	------	6.5	------	------
2 0 0	------	------	30	30	23	------	------	4.6	3.4	------	------	1.5	.98
2 0 1	------	------	------	95	50	------	------	11	6.1	------	------	2.6	1.6
2 0 2	------	------	------	------	95	------	------	------	10	------	------	------	2.3
2 1 0	------	------	------	240	62	------	------	24	7.3	------	------	4.1	1.9
2 1 1	------	------	------	------	130	------	------	------	13	------	------	------	2.8
2 1 2	------	------	------	------	210	------	------	------	21	------	------	------	4.1
2 2 0	------	------	------	------	240	------	------	------	24	------	------	------	4.0
2 2 1	------	------	------	------	700	------	------	------	70	------	------	------	8.1

Table 1-B.—*Most probable numbers per 100 cc of sample, planting various portions in not more than 3 dilutions*

Number of positive tubes in dilutions: Low Mid High	Combinations of portions planted in cubic centimeters: 3-10	3-10 1-1	4-10	4-10 1-1 1-0.1	4-10 2-1	4-10 4-1	5-10 1-1	5-10 1-1 1-0.1	5-10 2-1	5-50 1-10	5-50 1-10 1-1	5-50 2-10	5-100 1-50	5-100 1-50 1-10	5-100 2-50
0 0 1	-----	-----	-----	2.4	-----	-----	-----	2.0	-----	-----	0.38	-----	-----	0.18	-----
0 1 0	-----	3.3	-----	2.5	2.4	2.3	2.0	2.0	1.9	0.39	.39	0.38	0.19	.19	0.17
0 2 0	-----	-----	-----	-----	4.9	4.7	-----	-----	3.9	-----	-----	.77	-----	-----	.36
0 3 0	-----	-----	-----	-----	-----	7.1	-----	-----	-----	-----	-----	-----	-----	-----	-----
0 4 0	-----	-----	-----	-----	-----	9.5	-----	-----	-----	-----	-----	-----	-----	-----	-----
0 1 1	-----	-----	-----	4.9	-----	-----	-----	4.0	-----	-----	.78	-----	-----	.38	-----
1 0 0	4.1	3.9	2.9	2.8	2.7	2.6	2.2	2.2	2.1	.43	.43	.41	.20	.20	.18
1 0 1	-----	-----	-----	5.6	-----	-----	-----	4.4	-----	-----	.86	-----	-----	.40	-----
1 1 0	-----	8.1	-----	5.7	5.6	5.2	4.4	4.4	4.3	.88	.87	.84	.43	.42	.38
1 2 0	-----	-----	-----	-----	8.5	8.0	-----	-----	6.6	-----	-----	1.3	-----	-----	.61
1 3 0	-----	-----	-----	-----	-----	11	-----	-----	-----	-----	-----	-----	-----	-----	-----
1 4 0	-----	-----	-----	-----	-----	14	-----	-----	-----	-----	-----	-----	-----	-----	-----
1 1 1	-----	-----	-----	8.6	-----	-----	-----	6.7	-----	-----	1.3	-----	-----	.63	-----
2 0 0	11	10	6.9	6.7	6.5	6.1	5.0	5.0	4.9	.97	.97	.93	.45	.44	.41
2 0 1	-----	-----	-----	10	-----	-----	-----	7.5	-----	-----	1.5	-----	-----	.68	-----
2 1 0	-----	17	-----	10	10	9.4	7.6	7.6	7.5	1.5	1.5	1.4	.73	.71	.65
2 2 0	-----	-----	-----	-----	14	13	-----	-----	10	-----	-----	2.0	-----	-----	.93
2 3 0	-----	-----	-----	-----	-----	17	-----	-----	-----	-----	-----	-----	-----	-----	-----
2 4 0	-----	-----	-----	-----	-----	21	-----	-----	-----	-----	-----	-----	-----	-----	-----
2 1 1	-----	-----	-----	14	-----	-----	-----	10	-----	-----	2.0	-----	-----	.97	-----
3 0 0	-----	34	14	13	13	11	8.9	8.8	8.6	1.7	1.7	1.6	.79	.77	.69
3 0 1	-----	-----	-----	18	-----	-----	-----	12	-----	-----	2.3	-----	-----	1.1	-----
3 1 0	-----	-----	-----	19	18	16	12	12	12	2.4	2.4	2.3	1.2	1.1	1.0
3 2 0	-----	-----	-----	-----	25	13	-----	-----	16	-----	-----	3.0	-----	-----	1.4
3 3 0	-----	-----	-----	-----	-----	28	-----	-----	-----	-----	-----	-----	-----	-----	-----
3 4 0	-----	-----	-----	-----	-----	36	-----	-----	-----	-----	-----	-----	-----	-----	-----
3 1 1	-----	-----	-----	25	-----	-----	-----	16	-----	-----	3.1	-----	-----	1.5	-----
4 0 0	-----	-----	-----	36	30	24	15	15	15	2.9	2.9	2.7	1.3	1.3	1.1
4 0 1	-----	-----	-----	95	-----	-----	-----	20	-----	-----	3.8	-----	-----	1.7	-----
4 1 0	-----	-----	-----	240	71	39	21	21	20	4.1	4.0	3.7	1.9	1.8	1.6
4 2 0	-----	-----	-----	-----	-----	70	-----	-----	26	-----	-----	4.9	-----	-----	2.2
4 3 0	-----	-----	-----	-----	-----	140	-----	-----	-----	-----	-----	-----	-----	-----	-----
4 1 1	-----	-----	-----	-----	-----	-----	-----	27	-----	-----	5.2	-----	-----	2.4	-----
5 0 0	-----	-----	-----	-----	-----	-----	39	38	33	6.5	6.3	5.2	2.4	2.2	1.8
5 0 1	-----	-----	-----	-----	-----	-----	-----	96	-----	-----	11	-----	-----	3.1	-----
5 1 0	-----	-----	-----	-----	-----	-----	-----	240	72	-----	24	8.9	-----	4.7	2.8

TABLE 2.—*Most probable numbers per 100 cc of sample, planting 3, 4, or 5 portions in each of 3 dilutions in geometric series*

Number of positive tubes			Combinations of tubes planted		
10 cc	1 cc	0.1 cc	3-10 3-1 3-0.1	4-10 4-1 4-0.1	5-10 5-1 5-0.1
0	0	0	--------	--------	--------
0	0	1	3.0	2.3	1.8
0	0	2	6.0	4.5	3.6
0	0	3	9.0	6.8	5.4
0	0	4	--------	9.0	7.2
0	0	5	--------	--------	9.0
0	1	0	3.0	2.3	1.8
0	1	1	6.1	4.6	3.6
0	1	2	9.2	6.8	5.5
0	1	2	12	9.1	7.3
0	1	4	--------	11	9.1
0	1	5	--------	--------	11
0	2	0	6.2	4.6	3.7
0	2	1	9.3	6.9	5.5
0	2	2	12	9.2	7.4
0	2	3	16	12	9.2
0	2	4	--------	14	11
0	2	5	--------	--------	13
0	3	0	9.4	7.0	5.6
0	3	1	13	9.3	7.4
0	3	2	16	12	9.3
0	3	3	19	14	11
0	3	4	--------	16	13
0	3	5	--------	--------	15
0	4	0	--------	9.4	7.5
0	4	1	--------	12	9.4
0	4	2	--------	14	11
0	4	3	--------	17	13
0	4	4	--------	19	15
0	4	5	--------	--------	17
0	5	0	--------	--------	9.4
0	5	1	--------	--------	11
0	5	2	--------	--------	13
0	5	3	--------	--------	15
0	5	4	--------	--------	17
0	5	5	--------	--------	19
1	0	0	3.6	2.6	2.0
1	0	1	7.2	5.1	4.0
1	0	2	11	7.8	6.0
1	0	3	15	10	8.0
1	0	4	--------	13	10
1	0	5	--------	--------	12
1	1	0	7.3	5.2	4.0
1	1	1	11	7.9	6.1
1	1	2	15	11	8.1
1	1	3	19	13	10
1	1	4	--------	16	12
1	1	5	--------	--------	14
1	2	0	11	8.0	6.1
1	2	1	15	11	8.2
1	2	2	20	13	10
1	2	3	24	16	12
1	2	4	--------	19	15
1	2	5	--------	--------	17
1	3	0	16	11	8.3
1	3	1	20	14	10
1	3	2	24	16	13
1	3	3	29	19	15
1	3	4	--------	22	17
1	3	5	--------	--------	19
1	4	0	--------	14	11
1	4	1	--------	17	13
1	4	2	--------	20	15
1	4	3	--------	23	17
1	4	4	--------	26	19
1	4	5	--------	--------	22
1	5	0	--------	--------	13
1	5	1	--------	--------	15
1	5	2	--------	--------	17
1	5	3	--------	--------	19
1	5	4	--------	--------	22
1	5	5	--------	--------	24
2	0	0	9.1	6.0	4.5
2	0	1	14	9.1	6.8
2	0	2	20	12	9.1
2	0	3	26	16	12
2	0	4	--------	19	14
2	0	5	--------	--------	16
2	1	0	15	9.3	6.8
2	1	1	20	13	9.2
2	1	2	27	16	12
2	1	3	34	20	14
2	1	4	--------	23	17
2	1	5	--------	--------	19
2	2	0	21	13	9.3
2	2	1	28	16	12
2	2	2	35	20	14
2	2	3	42	24	17
2	2	4	--------	28	19
2	2	5	--------	--------	22
2	3	0	29	17	12
2	3	1	36	20	14
2	3	2	44	24	17
2	3	3	53	28	20
2	3	4	--------	32	22
2	3	5	--------	--------	25
2	4	0	--------	21	15
2	4	1	--------	25	17
2	4	2	--------	29	20
2	4	3	--------	33	23
2	4	4	--------	37	25
2	4	5	--------	--------	28
2	5	0	--------	--------	17
2	5	1	--------	--------	20
2	5	2	--------	--------	23
2	5	3	--------	--------	26
2	5	4	--------	--------	29
2	5	5	--------	--------	32

TABLE 2.—*Most probable numbers per 100 cc of sample, planting 3, 4, or 5 portions in each of 3 dilutions in geometric series*—Continued

Number of positive tubes	Combinations of tubes planted			Number of positive tubes	Combinations of tubes planted		Number of positive tubes	Combinations of tubes planted
10 cc 1 cc 0.1 cc	3-10 3-1 3-0.1	4-10 4-1 4-0.1	5-10 5-1 5-0.1	10 cc 1 cc 0.1 cc	4-10 4-1 4-0.1	5-10 5-1 5-0.1	10 cc 1 cc 0.1 cc	5-10 5-1 5-0.1
3 0 0	23	11	7.8	4 0 0	23	13	5 0 0	23
3 0 1	39	16	11	4 0 1	34	17	5 0 1	31
3 0 3	64	20	13	4 0 2	50	21	5 0 2	43
3 0 3	95	26	16	4 0 3	71	25	5 0 3	58
3 0 4	----------	31	20	4 0 4	95	30	5 0 4	76
3 0 5	----------	----------	23	4 0 5	----------	36	5 0 5	95
3 1 0	43	16	11	4 1 0	36	17	5 1 0	33
3 1 1	75	21	14	4 1 1	55	21	5 1 1	46
3 1 2	120	26	17	4 1 2	81	26	5 1 2	64
3 1 3	160	32	20	4 1 3	110	31	5 1 3	84
3 1 4	----------	38	23	4 1 4	140	36	5 1 4	110
3 1 5	----------	----------	27	4 1 5	----------	42	5 1 5	130
3 2 0	93	21	14	4 2 0	68	22	5 2 0	49
3 2 1	150	27	17	4 2 1	94	26	5 2 1	70
3 2 2	210	33	20	4 2 2	130	32	5 2 2	95
3 2 3	290	40	24	4 2 3	170	38	5 2 3	120
3 2 4	----------	47	27	4 2 4	210	44	5 2 4	150
3 2 5	----------	----------	31	4 2 5	----------	50	5 2 5	180
3 3 0	240	28	17	4 3 0	110	27	5 3 0	79
3 3 1	460	34	21	4 3 1	160	33	5 3 1	110
3 3 2	1,100	41	24	4 3 2	220	39	5 3 2	140
3 3 3	----------	48	28	4 3 3	280	45	5 3 3	180
3 3 4	----------	56	31	4 3 4	360	52	5 3 4	210
3 3 5	----------	----------	35	4 3 5	----------	59	5 3 5	250
3 4 0	----------	35	21	4 4 0	240	34	5 4 0	130
3 4 1	----------	43	24	4 4 1	390	40	5 4 1	170
3 4 2	----------	50	28	4 4 2	700	47	5 4 2	220
3 4 3	----------	59	32	4 4 3	1,400	54	5 4 3	280
3 4 4	----------	67	36	4 4 4	----------	62	5 4 4	350
3 4 5	----------	----------	40	4 4 5	----------	69	5 4 5	430
3 5 0	----------	----------	25	4 5 0	----------	41	5 5 0	240
3 5 1	----------	----------	29	4 5 1	----------	48	5 5 1	350
3 5 2	----------	----------	32	4 5 2	----------	56	5 5 2	540
3 5 3	----------	----------	37	4 5 3	----------	64	5 5 3	920
3 5 4	----------	----------	41	4 5 4	----------	72	5 5 4	1,600
3 5 5	----------	----------	45	4 5 5	----------	81	----------	----------

TABLE 3-A.—*Most probable numbers per 100 cc of sample, planting 5 portions in not more than 3 dilutions*

Number of positive tubes			Combinations of 10, 1, and 0.1 cc portions planted										
10 cc	1 cc	0.1 cc	0-0-5	0-1-4	0-2-3	0-3-2	0-4-1	1-1-3	1-2-2	1-3-1	2-1-2	2-2-1	3-1-1
0	0	1	220	74	45	32	25	9.0	8.3	7.7	4.7	4.5	3.2
0	0	2	510	150	91	65	--------	17	17	--------	9.5	--------	--------
0	0	3	920	240	140	--------	--------	27	--------	--------	--------	--------	--------
0	0	4	1,600	340	--------	--------	--------	--------	--------	--------	--------	--------	--------
0	1	0	--------	130	57	38	28	9.3	8.6	7.9	4.8	4.6	3.3
0	1	1	--------	330	120	77	57	19	17	16	9.7	9.3	6.5
0	1	2	--------	700	190	120	--------	28	26	--------	15	--------	--------
0	1	3	--------	1,400	280	--------	--------	38	--------	--------	--------	--------	--------
0	2	0	--------	--------	200	98	67	--------	18	17	--------	9.5	--------
0	2	1	--------	--------	450	160	100	--------	27	25	--------	14	--------
0	2	2	--------	--------	1,100	230	--------	--------	36	--------	--------	--------	--------
0	3	0	--------	--------	--------	280	130	--------	--------	26	--------	--------	--------
0	3	1	--------	--------	--------	710	190	--------	--------	35	--------	--------	--------
0	4	0	--------	--------	--------	--------	370	--------	--------	--------	--------	--------	--------
1	0	0	--------	--------	--------	--------	--------	22	17	14	6.4	6.0	3.9
1	0	1	--------	--------	--------	--------	--------	80	48	36	13	12	7.9
1	0	2	--------	--------	--------	--------	--------	170	95	--------	21	--------	--------
1	0	3	--------	--------	--------	--------	--------	260	--------	--------	--------	--------	--------
1	1	0	--------	--------	--------	--------	--------	150	61	42	14	13	8.0
1	1	1	--------	--------	--------	--------	--------	430	130	81	21	20	12
1	1	2	--------	--------	--------	--------	--------	1,100	210	--------	30	--------	--------
1	2	0	--------	--------	--------	--------	--------	--------	240	100	--------	21	--------
1	2	1	--------	--------	--------	--------	--------	--------	700	170	--------	29	--------
1	3	0	--------	--------	--------	--------	--------	--------	--------	340	--------	--------	--------
2	0	0	--------	--------	--------	--------	--------	--------	--------	--------	29	24	10
2	0	1	--------	--------	--------	--------	--------	--------	--------	--------	87	52	16
2	0	2	--------	--------	--------	--------	--------	--------	--------	--------	180	--------	--------
2	1	0	--------	--------	--------	--------	--------	--------	--------	--------	180	66	17
2	1	1	--------	--------	--------	--------	--------	--------	--------	--------	700	140	24
2	2	0	--------	--------	--------	--------	--------	--------	--------	--------	--------	300	--------
3	0	0	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	33
3	0	1	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	95
3	1	0	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	240

Table 3-B.—*Most probable numbers per 100 cc of sample, planting 5 portions in not more than 3 dilutions*

Number of positive tubes: 50 cc	10 cc	1 cc	Combinations of 50, 10, and 1.0 cc portions planted: 0-5-0	1-1-3	1-2-2	1-3-1	1-4-0	2-1-2	2-2-1	2-3-0	3-1-1	3-2-0	4-1-0	5-0-0
0	0	1	-------	1.6	1.4	1.2	-------	0.90	0.83	-------	0.62	-------	-------	-------
0	0	2	-------	3.2	2.8	-------	-------	1.8	-------	-------	-------	-------	-------	-------
0	0	3	-------	4.9	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------
0	0	4	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------
0	1	0	2.2	1.7	1.5	1.3	1.2	.94	.86	0.80	.64	0.61	0.49	-------
0	1	1	-------	3.5	3.0	2.7	-------	1.9	1.7	-------	1.3	-------	-------	-------
0	1	2	-------	5.3	4.6	-------	-------	2.8	-------	-------	-------	-------	-------	-------
0	1	3	-------	7.2	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------
0	2	0	5.1	-------	3.3	2.8	2.5	-------	1.8	1.7	-------	1.3	-------	-------
0	2	1	-------	-------	4.9	4.3	-------	-------	2.7	-------	-------	-------	-------	-------
0	2	2	-------	-------	6.7	-------	-------	-------	-------	-------	-------	-------	-------	-------
0	3	0	9.2	-------	-------	4.6	4.1	-------	-------	2.6	-------	-------	-------	-------
0	3	1	-------	-------	-------	6.3	-------	-------	-------	-------	-------	-------	-------	-------
0	4	0	16	-------	-------	-------	5.9	-------	-------	-------	-------	-------	-------	-------
1	0	0	-------	3.2	2.4	1.9	1.6	1.2	1.1	.97	.74	.70	.54	0.44
1	0	1	-------	8.5	5.5	4.2	-------	2.5	2.2	-------	1.5	-------	-------	-------
1	0	2	-------	17	9.7	-------	-------	3.8	-------	-------	-------	-------	-------	-------
1	0	3	-------	26	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------
1	1	0	-------	15	6.8	4.8	3.8	2.6	2.3	2.1	1.6	1.5	1.1	-------
1	1	1	-------	43	13	8.3	-------	4.1	3.6	-------	2.4	-------	-------	-------
1	1	2	-------	110	21	-------	-------	5.7	-------	-------	-------	-------	-------	-------
1	2	0	-------	-------	24	11	7.3	-------	3.9	3.4	-------	2.3	-------	-------
1	2	1	-------	-------	70	17	-------	-------	5.4	-------	-------	-------	-------	-------
1	3	0	-------	-------	-------	34	14	-------	-------	5.1	-------	-------	-------	-------
2	0	0	-------	-------	-------	-------	-------	4.5	3.5	2.9	1.9	1.8	1.3	1.0
2	0	1	-------	-------	-------	-------	-------	9.5	6.3	-------	3.1	-------	-------	-------
2	0	2	-------	-------	-------	-------	-------	18	-------	-------	-------	-------	-------	-------
2	1	0	-------	-------	-------	-------	-------	18	7.6	5.6	3.2	2.9	2.0	-------
2	1	1	-------	-------	-------	-------	-------	70	14	-------	4.6	-------	-------	-------
2	2	0	-------	-------	-------	-------	-------	-------	30	11	-------	4.3	-------	-------
3	0	0	-------	-------	-------	-------	-------	-------	-------	-------	5.4	4.3	2.5	1.8
3	0	1	-------	-------	-------	-------	-------	-------	-------	-------	10	-------	-------	-------
3	1	0	-------	-------	-------	-------	-------	-------	-------	-------	24	8.3	3.7	-------
4	0	0	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	6.1	3.2

TABLE 3-C.—*Most probable numbers per 100 cc of sample, planting 5 portions in not more than 3 dilutions*

Number of positive tubes			Combinations of 100, 50, and 10 cc portions planted											
100 cc	50 cc	10 cc	0-5-0	1-1-3	1-2-2	1-3-1	1-4-0	2-1-2	2-2-1	2-3-0	3-1-1	3-2-0	4-1-0	5-0-0
0	0	1		0.57	0.47	0.39		0.38	0.33		0.28			
0	0	2		1.2	.95			.77						
0	0	3		1.8										
0	0	4												
0	1	0	0.44	.65	.52	.43	0.36	.41	.35	0.31	.30	0.27	0.24	
0	1	1		1.4	1.1	.88		.84	.72		.61			
0	1	2		2.1	1.7			1.3						
0	1	3		3.0										
0	2	0	1.0		1.2	.97	.81		.78	.67		.58		
0	2	1			1.9	1.5			1.2					
0	2	2			2.7									
0	3	0	1.8			1.7	1.4			1.1				
0	3	1				2.4								
0	4	0	3.2				2.2							
1	0	0		.81	.61	.49	.41	.46	.39	.34	.33	.29	.25	0.22
1	0	1		1.8	1.3	1.0		.97	.81		.67			
1	0	2		3.1	2.1			1.5						
1	0	3		4.8										
1	1	0		2.5	1.5	1.2	.93	1.1	.89	.75	.72	.63	.54	
1	1	1		5.1	2.6	1.9		1.7	1.4		1.1			
1	1	2		11	4.0			2.5						
1	2	0			3.8	2.2	1.7		1.6	1.3		1.0		
1	2	1			6.0	3.4			2.2					
1	3	0				5.6	3.0			2.0				
2	0	0						1.4	1.0	.85	.81	.69	.59	.51
2	0	1						2.3	1.7		1.3			
2	0	2						3.7						
2	1	0						3.3	2.0	1.5	1.4	1.2	.98	
2	1	1						7.6	3.1		2.1			
2	2	0							5.0	2.7		1.9		
3	0	0									1.8	1.4	1.1	.92
3	0	1									2.8			
3	1	0									4.4	2.4	1.7	
4	0	0											2.2	1.6

The basic tables of M.P.N. values here presented may be expanded to meet a wide variety of combinations of portion plantings. Where such values are desired for any fraction or multiple of the dilution combination given, all that is necessary is to multiply the tabulated M.P.N. values of such combination by the quotient obtained by dividing the lowest dilution amount of the tabulated combination by the fraction or multiple required of this same lowest dilution. Thus, the M.P.N. values under the combination 2–10, 1–1, and 1–0.1 cc may be used to compute the values for the combination 2–100, 1–10, and 1–1 cc by multiplying each of the tabulated M.P.N. values by the common factor $\frac{10}{100}=0.1$; for the combination 2–0.1, 1–0.01, and 1–0.001 cc by using the multiplying factor $\frac{10}{0.1}=100$; or for the combination 2–50, 1–5, and 1–0.5 by using as the multiplier $\frac{10}{50}=0.2$, and so on.

Conversely, the M.P.N. value is the same for any multiple of a combination of portions and its corresponding multiple positive tube value as that given for the combination and positive tube result itself. Thus, the M.P.N. value in the tabulated combination 1–10, 5–1, and 1–0.1 cc where the positive tube result is 1—1—1 is 45 per 100 cc. This M.P.N. value is likewise correct for any multiple of this combination and its corresponding multiple of positive tube results, such as 2–10, 10–1, and 2–0.1 cc where the positive tube result is 2—2—2; for 3–10, 15–1, and 3–0.1 cc where the positive tube result is 3—3—3; and so on. Following this same principle, the tabulated values of the 4–10, 4–1, and 4–0.1 cc, for example, may be used to check the M.P.N. values of the 1–10, 1–1, and 1–0.1 cc, and the 2–10, 2–1, and 2–0.1 cc combinations which, for convenience, are given in the accompanying tables.

Where all tubes in all dilutions show growth or where all show no growth the result is, of course, indeterminate and no M.P.N. value can be computed. All that can be said is that the M.P.N. is greater or less than a certain value which may be computed on the assumption that the next dilution, if it had been planted, would have shown a change from positive to negative, or negative to positive, as the case may be. In any extended series of dilutions of a sample, the value of the M.P.N. is determined, practically, by the tubes of the dilutions in which the change is from positive to negative growth. Thus, in the series of dilutions with these results,

100 cc	10 cc	1 cc	0.1 cc	0.01 cc	0.001 cc	0.0001 cc
1+ 0–	2+ 0–	1+ 1–	0+ 2–	0+ 1–	0+ 1–	0+ 1–

the most probable number is defined practically entirely by the results of the 10-, 1-, and 0.1-cc tubes. Hence the M.P.N., which is 62 per 100 cc, may be obtained at once from table 1-A under the combination 2–10, 2–1, and 2–0.1 cc and opposite the positive result 2—1—0.

The slight degree to which the value of the M.P.N. is affected by extended dilutions beyond the range of the change from positive to negative tube results, is shown by the following example:

100 cc	50 cc	10 cc	1 cc	0.1 cc	M.P.N. per 100 cc
		4+ 1–			16
		4+ 1–	1–		15
		4+ 1–	1–	1–	15
		4+ 1–	2–		15
	1+	4+ 1–	1–		15
	1+	4+ 1–			16
1+	1+	4+ 1–			16
1+		4+ 1–			16
1+		4+ 1–	1–		15
1+		4+ 1–	5–	5–	13
5+		4+ 1–	5–		13

The futility of planting tubes in dilutions very far out of the range of this change is clearly indicated.

DISCUSSION

From a study of the M.P.N. values presented in these tables some conclusions of practical interest may be derived. For the purpose of simplifying this discussion, the M.P.N. values of "skip" or "inconsistent" analytical results in the various series are disregarded, although such results are entirely rational and any one of them may be

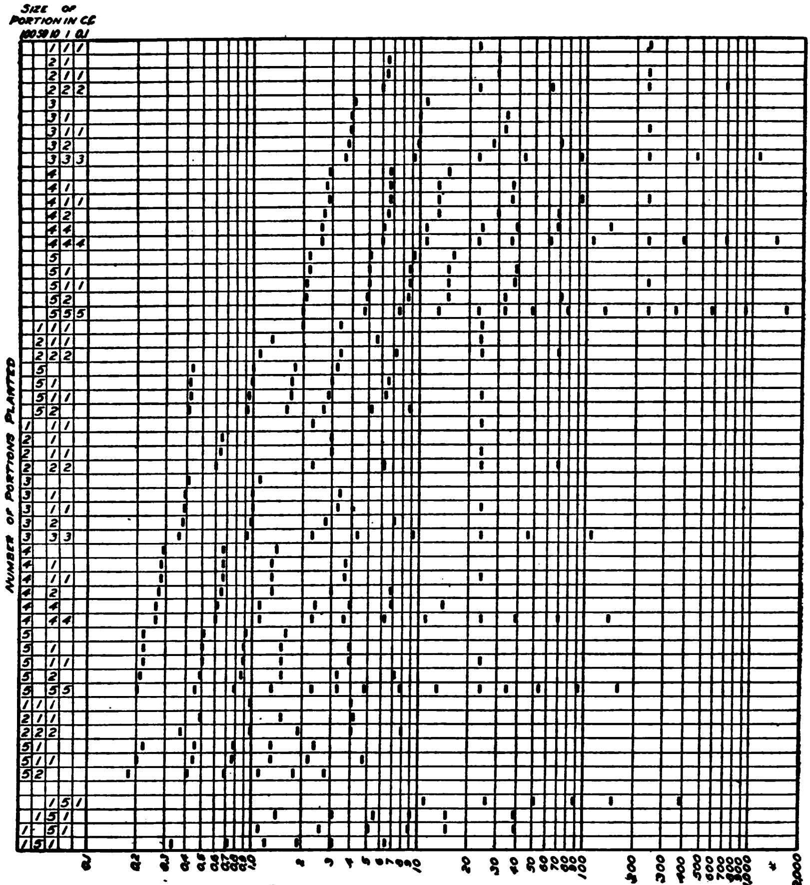

FIGURE 1.—Plot of most probable number values per 100 cc corresponding to analytical results (excluding anomalous or "skip" results) from the liquid media method of determination of the *coli-aerogenes* group, when designated numbers of specified portions of the sample are planted.

obtained at intervals of varying frequency. Omitting such results, the M.P.N. values of the various combinations of sample portions presented in heavy type in the tables are plotted in a logarithmic scale in figures 1 and 2.

It will be observed that the lowest M.P.N. values are quite definitely limited by the size of the largest portion planted, are limited to a lesser degree by the number of portions planted at this dilution, and are changed not at all by an increase in the number of portions of smaller amount than this largest portion. Thus, in figure 1, the lowest M.P.N. value obtainable from one 10-cc positive tube, in any series in which 10 cc is the largest portion of sample planted, ranges from 23 per 100 cc, where the series is 1—1—1, to 2.0 per 100 cc in the series 5—5—5. However, increases in the number of portions planted at the various dilutions do tend to measure more accurately the M.P.N. value of the sample within the limits of the range, because

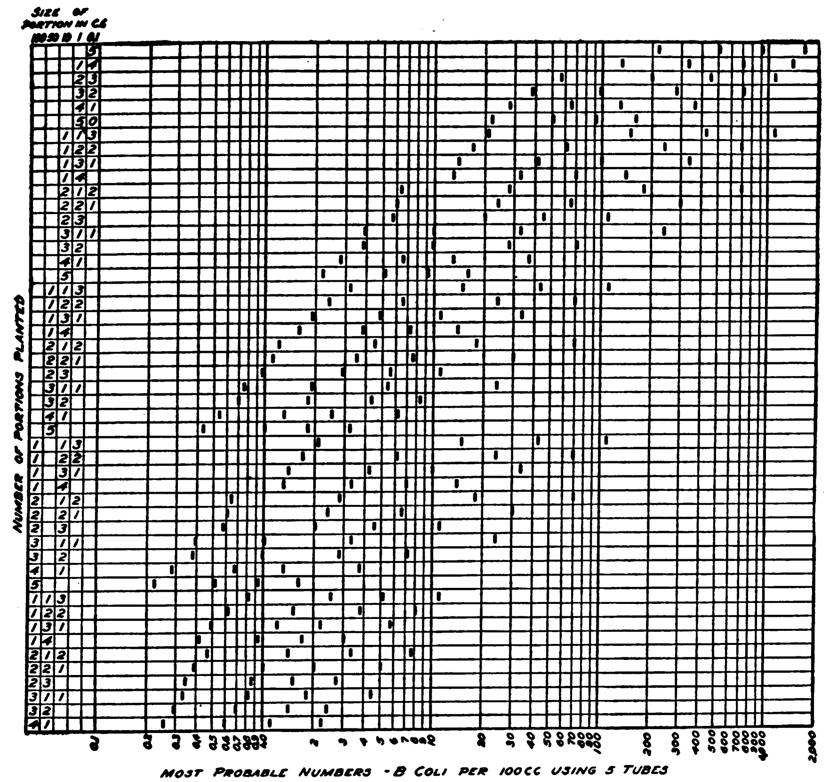

FIGURE 2.—Plot of most probable number values per 100 cc corresponding to analytical results (excluding anomalous or "skip" results) from the liquid media method of determination of the *coli-aerogenes* group when five portions of designated size of the sample are planted.

the possible values obtainable within the range are always one less in number than the number of tubes planted. For example, in the series of 1–10, 1–1, and 0.1 cc, only two values are possible (either 23 or 240 per 100 cc), whereas in the series 5–10, 5–1, 5–0.1 cc any one of 14 values between 2.0 and 1,600 per 100 cc are possible, depending upon the combination of positive and negative tube results. Therefore, when the range of bacterial density in the sample under examination can be reasonably estimated, it is advantageous to plant the greater number of portions in the range corresponding to this estimate, rather than equal numbers of portions in an indiscriminate range.

This principle is particularly applicable to the bacteriological analysis of drinking water supplies. Here the upper limit is generally required to conform to the Treasury Department standard of 1.05 *coli-aerogenes* group organisms per 100 cc. Yet no reasonable number of 10-cc portions of a sample examined will measure the content much below two such organisms per 100 cc. In other words, as Reed (2) points out, the measuring stick is too coarse for this particular purpose. Consequently, when 10-cc portions are planted, the water purification plant operator has no means of knowing at any time how closely the bacterial content of the finished water is approaching this upper limit, and his bacteriological test is not of the maximum value to him that it should be. This difficulty can be overcome readily, however, if, instead of 10 cc portions, 100 cc portions of the sample are planted.

As shown by the tables, the range of, for example, five 100-cc tubes will extend from 0.22 to 1.6 organisms per 100 cc; or, if five 50-cc tubes are inoculated, from 0.44 to 3.2 per 100 cc instead of from 2.2 to 16 per 100 cc when five 10-cc tubes are planted. It would appear highly desirable, therefore, to increase the size of the portion examined in order to increase the value of this routine test. Such procedure offers no difficulty in laboratory technique, the only requirement being larger tubes or containers for inoculation, larger quantities of media, and slightly greater incubator space. Double-strength broth—about 75 cc for the 100-cc portion—in the inoculation tube is usually satisfactory.

In routine laboratory work there is usually a definite, practicable limit to the number of tubes that can be examined. It is of particular interest, therefore, that the greatest possible return be obtained from the analytical results. Careful selection of the series of dilutions employed will increase the usefulness of the test and at the same time reduce the volume of routine laboratory work. It may be assumed that, for routine work, five portions of each sample are about all that can be expected to be inoculated. Upon this assumption, all the most probable numbers of all possible series of combinations employing five tubes in not more than three dilutions are presented in tables 3-A, 3-B, and 3-C. In general, a careful selection of the combination from these series will meet most routine requirements. In special cases where the bacterial density of the sample cannot be estimated, planting of one or more portions at each dilution in an extended series is perhaps the preferable procedure and then, for purpose of interpretation, discarding the positive and negative results, excepting only those immediately above and below the point of change in sign. Thus, the series of the 5—1, 5—1—1 or

1—5—1 combinations may be extended by single tubes in geometric series in either higher or lower dilutions and the result readily interpreted by means of the tables, regardless of the dilution in which the change may occur. Figure 1 shows graphically, for example, how the combination 1—5—1 in various dilutions may be adapted to cover the entire range of bacterial density of samples.

To aid the judgment in the selection of the proper combination of portions in water purification practice, experience with the waters dealt with is the best guide. Streeter (3) has shown that for the various stages of the treatment process, comprising coagulation, rapid sand filtration, and chlorination, certain concentrations of *coli-aerogenes* group organisms are about limiting numbers that can be expected to be present if the final effluent is to conform to the Treasury Department standard for drinking water. These limiting numbers are given in the first column of the following summary, opposite which are set down suggested combinations of sample portions for examination which will cover the stated density range:

Water	Limiting concentration M.P.N. per 100 cc	Combination of portions examined	Range measured M.P.N. per 100 cc
Raw water	9,000	2-0.1, 3-0.01 cc	570 to 11,000.
Applied water	3,700	4-0.1, 1-0.01 cc	280 to 3,700.
Filtered water	35	3-100, 2-10 cc	3.8 to 71.
Chlorinated water	1.05	5-100 cc	0.22 to 1.6.

These combinations are given only as an illustration of the selection method. Other combinations in the accompanying tables may be chosen to conform more closely to specific conditions or where it is deemed advisable to extend the range either above or below a certain estimated density of *coli-aerogenes* organisms. In general, where the bacterial density of a water changes little from day to day, a properly selected series employing a total of five portions of sample will meet most routine requirements and afford a well-defined picture of the *coli-aerogenes* content.

REFERENCES

(1) Hoskins, J. K.: The most probable numbers of *B. coli* in water analysis. Jour. Am. Water Works Assoc., **25**:867-877 (June 1933).

(2) Reed, Lowell J.: Drinking water standards. Appendix III. *B. coli* densities as determined from various types of samples. Pub. Health Rep., **40**:693-721 (April 10, 1925). Reprint no. 1029.

(3) Streeter, H. W.: The bacterial efficiency of certain intermediate stages of water treatment. Public Works. **64**:17-20 (December 1933).

COURT DECISION ON PUBLIC HEALTH

Conviction for unlawful possession of "mariguana" sustained.—(Utah Supreme Court; *State* v. *Navaro*, 26 P.(2d) 955; decided Nov. 17, 1933.) A Utah statute made it unlawful, among other things, for a person "to have in possession any cocaine, opium, morphine, codeine, heroin, peyote (mescal button), alpha eucaine, beta eucaine, nova caine, flowering tops and leaves, extracts, tinctures, and other narcotic preparations of hemp or loco weed, (cannabis sativa, Indian hemp), mariguana, or chloral hydrate, or any of the salts, derivatives, or compounds of the foregoing substances, or any preparation or compound containing any of the foregoing substances, or their salts, derivatives, or compounds". Under the statute, possession of the drugs named was lawful under certain circumstances, such as, for example, upon the written order or prescription of a physician. The defendant was convicted of unlawfully possessing mariguana. The evidence showed that he was stopped on a public street by two police officers. One of them drew from the defendant's shirt pocket a package containing 10 cigarettes done up in brown papers. The officers testified that the defendant said that the package belonged to him and that it contained mariguana. The defendant denied making such statements. The city chemist of Salt Lake City examined the package's contents and testified that he found that the cigarettes contained American cannabis, or mariguana.

On appeal to the supreme court, the defendant contended that the statute did not prohibit possession of mariguana itself but of the flowering tops and leaves of mariguana, the tincture, extract, or other preparations of mariguana, and that the information, in order to charge an offense under the statute, should have charged unlawful "possession of the flowering tops and leaves of mariguana" instead of directly charging unlawful "possession of marijuana". This view was predicated on the grammatical construction of the pertinent sentence in the statute and on the definition of the word "marijuana", which the defendant claimed meant a plant and not a drug.

The supreme court said that it would seem that "mariguana", when used without qualifying or modifying words, indicated the product or preparation consisting of the flowering tops, leaves, and seeds of the plant rather than either the whole plant or the fibrous stalks thereof. Further along in the opinion the court stated that it thought that the preponderant use of the word was clearly with reference to the product used for smoking. "Such use is so frequent and common that no one can misunderstand when the statute prohibits its unauthorized possession or sale as a drug. The information in this case charges the unlawful possession of mariguana in the language of the statute and that is sufficient."

Respecting the grammatical construction of the pertinent sentence, the defendant claimed that the words "flowering tops and leaves, extracts, tinctures, and preparations" were modified by the words "hemp, loco weed, (cannabis sativa, Indian hemp), mariguana, and chloral hydrate". But the court disagreed with this view, saying that, if this contention were correct, "the statute must be construed to prohibit possession of the flowering tops and leaves of chloral hydrate as well as of mariguana". This, however, was stated by the court to be an impossible construction because chloral hydrate was unquestionably not a plant but a drug.

In rejecting another contention of the defendant that it was incumbent on the State to produce evidence to prove the negative allegations of the information, the court quoted from 49 C.J. 1053 as follows:

> Where the statute relating to poisons or narcotic drugs contains exceptions, a defendant desiring to avail himself of any of them by way of defense must show that he comes within its intent. Thus the burden is upon one accused of illegal possession to show that his possession was lawful under a proviso or exception of the statute under which he is being prosecuted, or, where the animus possidendi is an element of the offense, to show honest ignorance of the fact of possession.

DEATHS DURING WEEK ENDED MAR. 3, 1934

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

	Week ended Mar. 3, 1934	Corresponding week, 1933
Data from 86 large cities of the United States:		
Total deaths	9,180	8,260
Deaths per 1,000 population, annual basis	12.8	11.5
Deaths under 1 year of age	657	617
Deaths under 1 year of age per 1,000 estimated live births	61	[1] 53
Deaths per 1,000 population, annual basis, first 9 weeks of year	12.7	12.5
Data from industrial insurance companies:		
Policies in force	67,566,995	68,947,917
Number of death claims	15,836	15,423
Death claims per 1,000 policies in force, annual rate	12.2	11.7
Death claims per 1,000 policies, first 9 weeks of year, annual rate	10.9	11.4

[1] Data for 81 cities.

PREVALENCE OF DISEASE

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

UNITED STATES

CURRENT WEEKLY STATE REPORTS

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

Reports for Weeks Ended Mar. 10, 1934, and Mar. 11, 1933

Cases of certain communicable diseases reported by telegraph by State health officers for weeks ended Mar. 10, 1934, and Mar. 11, 1933

Division and State	Diphtheria		Influenza		Measles		Meningococcus meningitis	
	Week ended Mar. 10, 1934	Week ended Mar. 11, 1933	Week ended Mar. 10, 1934	Week ended Mar. 11, 1933	Week ended Mar. 10, 1934	Week ended Mar. 11, 1933	Week ended Mar. 10, 1934	Week ended Mar. 11, 1933
New England States:								
Maine				1	1	2	0	0
New Hampshire			2	3	126		0	0
Vermont		1			54	21	0	0
Massachusetts	19	28		11	2,356	355	2	0
Rhode Island	4	2		5	9		0	0
Connecticut	2	5	2	7	36	328	1	2
Middle Atlantic States:								
New York	53	70	[1] 22	[1] 30	1,330	3,519	4	7
New Jersey	15	23	24	34	547	1,594	0	2
Pennsylvania	54	78			3,063	1,242	7	7
East North Central States:								
Ohio	17	43	21	215	888	529	0	0
Indiana	22	42	64	83	750	85	1	3
Illinois	23	31	39	68	1,473	276	3	19
Michigan	18	19	3	9	95	1,531	3	7
Wisconsin	5	5	66	137	1,278	412	2	1
West North Central States:								
Minnesota	4	5	2	2	315	1,102	0	0
Iowa [2]	1	12	11		158	14	1	2
Missouri	35	27	188	17	1,354	243	2	4
North Dakota	1	7	29	26	129	18	0	1
South Dakota	3	5			837	6	0	0
Nebraska	1	7		3	50	22	1	1
Kansas	11	4		6	256	237	0	4
South Atlantic States:								
Delaware		1			269	2	0	0
Maryland [3]	7	8	21	70	670	6	0	0
District of Columbia	10	3	1	3	555	5	0	0
Virginia	26	18			1,334	647	2	2
West Virginia	14	12	83	43	48	166	0	0
North Carolina	25	12	49	105	2,822	371	1	2
South Carolina	7	5	871	918	654	204	0	0
Georgia [3]	16	8		445	1,817	29	2	2
Florida	6	7	2	13	279	25	0	0
East South Central States:								
Kentucky	27	13	113	77	635	67	0	2
Tennessee	8	9	132	85	1,180	33	8	8
Alabama [3]	23	15	102	113	875	41	2	1
Mississippi [3]	3	7					0	1
West South Central States:								
Arkansas	7	4	105	49	492	119	0	2
Louisiana	35	23	16	56	185	40	0	1
Oklahoma [4]	15	21	124	141	490	71	0	0
Texas [3]	106	48	724	135	1,131	710	3	1
Mountain States:								
Montana	8		26	15	57	94	1	0
Idaho	1	1		3	19	94	1	0
Wyoming				1	77	1	0	0
Colorado	6	2		47	235	3	0	7
New Mexico	9	11	2	2	58	12	0	1
Arizona	1	3	17		38	34	0	0
Utah [3]	1	1		5	624	4	0	2

See footnotes at end of table.

Cases of certain communicable diseases reported by telegraph by State health officers for weeks ended Mar. 10, 1934, and Mar. 11, 1933—Continued

Division and State	Diphtheria		Influenza		Measles		Meningococcus meningitis	
	Week ended Mar. 10, 1934	Week ended Mar. 11, 1933	Week ended Mar. 10, 1934	Week ended Mar. 11, 1933	Week ended Mar. 10, 1934	Week ended Mar. 11, 1933	Week ended Mar. 10, 1934	Week ended Mar. 11, 1933
Pacific States:								
Washington	2	4	2		173	3	0	0
Oregon	3	3	81	73	107	108	0	0
California	39	49	27	107	1,491	985	2	3
Total	693	702	2,971	3,163	31,420	15,410	49	95

Division and State	Poliomyelitis		Scarlet fever		Smallpox		Typhoid fever	
	Week ended Mar. 10, 1934	Week ended Mar. 11, 1933	Week ended Mar. 10, 1934	Week ended Mar. 11, 1933	Week ended Mar. 10, 1934	Week ended Mar. 11, 1933	Week ended Mar. 10, 1934	Week ended Mar. 11, 1933
New England States:								
Maine	0	0	10	14	0	0	1	2
New Hampshire	0	0	7	50	0	0	0	1
Vermont	0	0	6	15	0	0	0	0
Massachusetts	1	1	275	393	0	0	1	0
Rhode Island	0	0	23	25	0	0	0	0
Connecticut	0	0	71	115	0	0	1	1
Middle Atlantic States:								
New York	1	0	874	1,009	0	0	10	10
New Jersey	0	0	216	382	0	0	2	4
Pennsylvania	1	0	798	956	0	0	9	9
East North Central States:								
Ohio	0	0	826	967	1	2	2	8
Indiana	0	0	261	197	1	1	2	1
Illinois	0	1	654	471	3	26	6	1
Michigan	0	1	801	558	6	2	3	4
Wisconsin	1	0	308	160	10	9	2	1
West North Central States:								
Minnesota	0	0	66	88	5	0	0	0
Iowa [2]	0	0	85	53	18	49	1	1
Missouri	0	0	118	95	0	0	2	1
North Dakota	0	0	13	21	0	5	0	1
South Dakota	0	1	12	24	10	0	0	3
Nebraska	1	0	11	37	0	1	0	0
Kansas	0	0	97	58	1	0	1	2
South Atlantic States:								
Delaware	0	0	11	15	0	0	0	0
Maryland [3]	0	1	95	113	0	0	2	14
District of Columbia	0	0	17	21	0	0	0	0
Virginia	2	1	33	59	0	4	3	8
West Virginia	0	0	77	31	0	0	2	4
North Carolina	0	1	37	31	0	0	0	3
South Carolina	0	0	6	8	0	0	6	0
Georgia [3]	0	0	4	9	0	14	9	3
Florida	0	1	2	5	0	0	1	0
East South Central States:								
Kentucky	0	0	60	50	0	0	6	9
Tennessee	0	1	26	49	9	0	3	5
Alabama [3]	0	0	10	14	0	1	0	1
Mississippi [2]	0	1	5	5	0	0	3	5
West South Central States:								
Arkansas	0	0	5	19	2	22	4	1
Louisiana	0	0	22	18	1	0	17	5
Oklahoma [4]	0	1	17	31	0	9	7	0
Texas [3]	0	0	120	44	39	9	10	8
Mountain States:								
Montana	0	0	17	16	0	1	0	7
Idaho	0	0	2	0	16	4	0	0
Wyoming	0	0	3	4	0	0	0	0
Colorado	0	0	24	43	2	1	0	1
New Mexico	1	0	24	8	1	0	0	0
Arizona	0	0	13	8	0	0	0	0
Utah [2]	0	0	7	19	4	0	0	1
Pacific States:								
Washington	3	0	83	52	10	4	5	3
Oregon	0	0	38	10	0	2	2	2
California	2	2	247	217	4	39	11	9
Total	13	13	6,537	6,587	143	205	134	139

[1] New York City only.
[2] Week ended earlier than Saturday.
[3] Typhus fever, week ended Mar. 10, 1934, 15 cases, as follows: Georgia, 3; Alabama, 6; Texas, 6.
[4] Exclusive of Oklahoma City and Tulsa.

SUMMARY OF MONTHLY REPORTS FROM STATES

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

State	Meningococcus meningitis	Diphtheria	Influenza	Malaria	Measles	Pellagra	Poliomyelitis	Scarlet fever	Smallpox	Typhoid fever
January 1934										
Kansas	4	46	24		192		1	588	12	8
Mississippi	1	65	5,092	1,674	2,493	196	2	86	7	15
Nevada		2	22		24		2	11	3	1
February 1934										
Arkansas	5	36	317	25	2,240	15	0	41	24	6
Connecticut		16	32		160		0	208	0	2
Delaware		10	3		782		0	65	0	0
District of Columbia	1	34	12		1,572	1	1	73	0	0
Maine	1	4	11		8		0	80	0	6
Massachusetts	6	27		1	8,637	1	0	972	0	9
Nebraska	1	29	95		305		0	93		2
Vermont		3			238		1	58	0	3
Wyoming	1	3		1	194		1	21	1	0

January 1934

	Cases
Chicken pox:	
Kansas	940
Mississippi	730
Nevada	6
Dengue:	
Mississippi	3
Dysentery:	
Mississippi (amoebic)	26
German measles:	
Kansas	15
Hookworm disease:	
Mississippi	344
Impetigo contagiosa:	
Kansas	2
Lethargic encephalitis:	
Kansas	7
Mumps:	
Kansas	561
Mississippi	330
Ophthalmia neonatorum:	
Kansas	2
Puerperal septicemia:	
Mississippi	32
Rabies in animals:	
Mississippi	5
Scabies:	
Kansas	2
Tetanus:	
Kansas	1
Trachoma:	
Mississippi	4
Undulant fever:	
Kansas	6
Vincent's infection:	
Kansas	2
Whooping cough:	
Kansas	517
Mississippi	1,554
Nevada	4

February 1934

	Cases
Anthrax:	
Delaware	1
Massachusetts	2
Nebraska	1

February 1934—Contd.

	Cases
Chicken pox:	
Arkansas	76
Connecticut	415
Delaware	67
District of Columbia	94
Maine	291
Massachusetts	1,157
Nebraska	229
Vermont	228
Wyoming	74
Conjunctivitis:	
Connecticut	1
Wyoming	3
Dysentery:	
Connecticut (amoebic)	1
Delaware	1
Maine (amoebic)	1
Massachusetts (amoebic)	4
Massachusetts (bacillary)	2
Nebraska (amoebic)	1
German measles:	
Connecticut	9
Maine	65
Massachusetts	57
Wyoming	32
Lead poisoning:	
Connecticut	1
Massachusetts	1
Lethargic encephalitis:	
Massachusetts	3
Nebraska	1
Mumps:	
Arkansas	121
Connecticut	441
Delaware	18
Maine	13
Massachusetts	488
Nebraska	110
Vermont	32
Wyoming	27

February 1934—Contd.

	Cases
Ophthalmia neonatorum:	
Arkansas	1
Massachusetts	46
Paratyphoid fever:	
Maine	1
Rabies in animals:	
Connecticut	2
Massachusetts	22
Rocky Mountain spotted fever:	
Wyoming	3
Septic sore throat:	
Connecticut	8
Maine	3
Massachusetts	21
Nebraska	4
Wyoming	4
Trachoma:	
Arkansas	5
Connecticut	1
Massachusetts	1
Trichinosis:	
Connecticut	2
Massachusetts	4
Undulant fever:	
Arkansas	1
Connecticut	2
Delaware	1
Maine	1
Vincent's infection:	
Maine	4
Whooping cough:	
Arkansas	94
Connecticut	154
Delaware	60
District of Columbia	102
Maine	326
Massachusetts	1,273
Nebraska	212
Vermont	58
Wyoming	17

CASES OF VENEREAL DISEASES REPORTED FOR JANUARY 1934

This statement is published monthly for the information of health officers in order to furnish current data as to the prevalence of the venereal diseases. The figures are taken from reports received from State health officers. They are preliminary and are, therefore, subject to correction. It is hoped that the publication of these reports will stimulate more complete reporting of these diseases.

State	Syphilis		Gonorrhea	
	Cases reported during month	Monthly case rates per 10,000 population	Cases reported during month	Monthly case rates per 10,000 population
Alabama [b]				
Arizona	21	0.48	40	0.92
Arkansas [c]	2		12	.06
California	1,949	3.43	1,559	2.75
Colorado [a]				
Connecticut [b]				
Delaware	105	4.40	22	.92
District of Columbia	139	2.85	98	2.01
Florida	567	3.86	72	.49
Georgia	430	1.48	401	1.38
Idaho	0	0	0	0
Illinois	1,215	1.59	1,120	1.47
Indiana	133	.41	122	.38
Iowa [b]				
Kansas	73	.39	53	.28
Kentucky	238	.91	357	1.37
Louisiana	170	.81	114	.54
Maine	57	.71	49	.61
Maryland	283	1.74	192	1.18
Massachusetts	372	.88	516	1.21
Michigan [b]				
Minnesota	270	1.05	312	1.22
Mississippi	890	4.43	1,447	7.20
Missouri [b]				
Montana [c]	20	.37	12	.22
Nebraska	36	.26	101	.73
Nevada [a]				
New Hampshire	14	.30	30	.64
New Jersey	725	1.79	299	.74
New Mexico [c]	44	1.04	44	1.04
New York	5,250	4.17	1,329	1.06
North Carolina	963	3.04	424	1.34
North Dakota	15	.22	39	.57
Ohio [b]				
Oklahoma [c]	134	.56	163	.68
Oregon [b]				
Pennsylvania	316	.33	283	.29
Rhode Island	82	1.19	43	.63
South Carolina [c]	401	2.31	599	3.44
South Dakota	11	.16	33	.48
Tennessee	1,024	3.91	553	2.12
Texas [b]				
Utah [a]				
Vermont	26	.72	29	.81
Virginia [b]				
Washington	116	.74	221	1.41
West Virginia [b]				
Wisconsin [d]	26	.09	163	.55
Wyoming	4	.18	4	.18
Total	16,121	1.83	10,855	1.23

[a] Not reporting.
[b] Have been reporting regularly but no report received for current month.
[c] Incomplete.
[d] Only cases of syphilis in the infectious stage are reported.

NOTE.—Surveys in which all medical sources have been contacted in representative communities throughout the United States have revealed that the monthly rate per 10,000 population is 6.6 for syphilis and 10.2 for gonorrhea.

WEEKLY REPORTS FROM CITIES

City reports for week ended Mar. 3, 1934

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

State and city	Diphtheria cases	Influenza		Measles cases	Pneumonia deaths	Scarlet fever cases	Smallpox cases	Tuberculosis deaths	Typhoid fever cases	Whooping cough cases	Deaths, all causes
		Cases	Deaths								
Maine:											
Portland	0	1	0	1	7	1	0	0	0	13	33
New Hampshire:											
Concord	0	------	0	29	1	0	0	1	0	0	21
Nashua	0	------	0	1	0	2	0	0	0	2	-------
Vermont:											
Barre	0	------	0	0	0	0	0	1	0	0	1
Burlington	0	------	0	0	0	2	0	0	0	7	6
Massachusetts:											
Boston	1	------	1	375	40	52	0	11	0	55	255
Fall River	0	------	0	0	2	1	0	2	2	2	28
Springfield	0	------	0	2	2	5	0	1	0	4	37
Worcester	2	------	0	35	5	8	0	0	0	5	49
Rhode Island:											
Pawtucket	1	------	0	2	0	0	0	0	0	0	-------
Providence	3	------	0	7	7	10	0	1	0	19	72
Connecticut:											
Bridgeport	1	4	2	2	3	10	0	2	0	3	27
Hartford	1	------	0	0	6	10	0	1	0	1	44
New Haven	0	------	1	4	2	1	0	0	0	0	31
New York:											
Buffalo	1	------	1	258	15	20	0	7	0	12	156
New York	40	32	17	62	213	296	0	106	6	113	1,737
Rochester	5	1	1	3	6	37	0	2	0	5	58
Syracuse	0	------	0	3	8	8	0	0	0	46	52
New Jersey:											
Camden	0	2	0	148	4	4	0	0	0	1	43
Newark	0	4	0	6	12	27	0	9	0	23	119
Trenton	0	1	1	58	0	22	0	4	0	2	56
Pennsylvania:											
Philadelphia	2	16	7	1,418	77	118	0	36	0	38	618
Pittsburgh	5	6	8	75	40	35	0	7	0	50	226
Reading	0	------	0	4	3	8	0	0	0	6	18
Scranton	0	------	0	0	0	6	0	0	0	10	-------
Ohio:											
Cincinnati	2	------	4	144	13	41	0	7	0	15	134
Cleveland	6	63	6	29	31	115	0	7	0	101	213
Columbus	4	1	1	6	6	38	0	4	0	16	86
Toledo	1	------	0	126	9	45	0	3	0	66	74
Indiana:											
Fort Wayne	2	------	1	6	1	10	0	0	0	1	21
Indianapolis	1	------	2	230	17	29	0	6	0	35	-------
South Bend	0	------	0	0	3	10	0	0	0	0	22
Terre Haute	0	------	1	3	1	0	0	0	0	5	22
Illinois:											
Chicago	0	14	3	64	66	302	0	31	0	195	717
Cicero	0	------	0	0	0	0	0	0	0	0	3
Springfield	0	2	0	78	6	0	0	0	0	13	31
Michigan:											
Detroit	8	2	8	17	40	181	0	16	0	113	261
Flint	0	------	1	3	6	99	0	1	1	0	29
Grand Rapids	0	------	0	1	3	25	0	0	0	5	36
Wisconsin:											
Kenosha	0	------	0	0	0	27	0	1	0	0	7
Milwaukee	1	1	1	9	0	103	0	4	0	109	100
Racine	1	------	0	0	2	10	1	0	0	11	13
Superior	0	------	0	0	0	0	0	0	0	2	6
Minnesota:											
Duluth	0	------	2	0	1	2	0	1	0	3	28
Minneapolis	8	------	1	4	5	12	0	2	0	18	115
St. Paul	0	------	0	1	10	9	2	5	0	5	70
Iowa:											
Des Moines	0	------	-------	0	-------	17	0	-------	0	0	34
Sioux City	1	------	-------	12	-------	2	0	0	0	0	-------
Waterloo	0	------	-------	0	-------	0	0	-------	0	9	-------
Missouri:											
Kansas City	2	------	1	10	11	33	0	6	0	62	85
St. Joseph	4	------	0	9	9	4	0	0	0	0	31
St. Louis	25	3	1	468	18	16	0	7	1	63	243

City reports for week ended Mar. 3, 1934—Continued

State and city	Diphtheria cases	Influenza		Measles cases	Pneumonia deaths	Scarlet fever cases	Smallpox cases	Tuberculosis deaths	Typhoid fever cases	Whooping cough cases	Deaths, all causes
		Cases	Deaths								
North Dakota:											
Fargo	0		0	124	0	0	0	1	0	2	8
South Dakota:											
Aberdeen	0		0	3	0	0	0	0	0	2	
Sioux Falls	0		0	19	0	0	0	0	0	0	8
Nebraska:											
Omaha	2		1	184	6	6	1	3	0	18	64
Kansas:											
Topeka	0		0	1	3	5	0	1	0	5	19
Wichita	1		0	11	6	3	0	2	1	5	32
Delaware:											
Wilmington	1		0	53	1	3	0	0	0	3	30
Maryland:											
Baltimore	8	3	1	411	47	34	0	7	0	136	235
Cumberland	0	2	0	0	2	6	0	0	0	2	14
Frederick											
District of Columbia:											
Washington	7	1	1	514	18	16	0	13	1	22	178
Virginia:											
Lynchburg	1		1	0	2	1	0	4	0	0	16
Richmond	2	2	1	47	7	3	0	0	1	6	70
Roanoke	2		1	0	1	1	0	1	0	0	23
West Virginia:											
Charleston	0		0	0	4	0	0	1	0	0	14
Huntington	2		0	0	0	5	0	0	0	0	
Wheeling	0		0	0	8	6	0	0	0	4	21
North Carolina:											
Raleigh	0		0	26	4	0	0	0	0	6	16
Wilmington	0		0	0	1	0	0	0	0	0	10
Winston-Salem	2	2	1	81	5	1	0	1	0	3	17
South Carolina:											
Charleston	0	59	1	27	4	0	0	0	0	1	29
Columbia											
Greenville	0		0	3	3	1	0	0	0	8	12
Georgia:											
Atlanta	5	27	3	296	12	4	0	4	0	2	93
Brunswick	0		0	177	1	0	0	0	0	0	3
Savannah	1	46	3	74	6	2	0	2	1	0	44
Florida:											
Miami	0		0	4	3	0	0	1	0	4	38
Tampa	2		0	19	0	0	0	0	0	0	18
Kentucky:											
Ashland											
Lexington	1		0	1	3	0	0	0	0	5	19
Tennessee:											
Memphis	0		2	386	12	0	0	3	0	8	93
Nashville	0		1	108	11	2	0	0	0	13	51
Alabama:											
Birmingham	0	10	3	70	8	2	0	5	0	1	69
Mobile	1	1	1	11	1	0	0	0	0	0	16
Montgomery	1	1		16		1	0		0	3	
Arkansas:											
Fort Smith	0			36		0	0		0	0	
Little Rock	0		0	115	4	0	0	2	0	1	9
Louisiana:											
New Orleans	21	5	5	17	19	8	0	13	1	0	154
Shreveport	1		1	5	7	3	0	1	1	4	31
Texas:											
Dallas	9	4	4	0	11	16	1	1	0	0	67
Fort Worth	1		0	0	12	7	0	0	0	8	50
Galveston	0		0	0	3	2	0	0	0	0	12
Houston	12		0	11	11	12	3	2	0	0	70
San Antonio	0		1	7	10	2	0	9	0	6	52
Montana:											
Billings	0		0	0	0	1	0	0	0	0	4
Great Falls	0		0	2	3	0	0	0	0	0	11
Helena	0		0	0	0	0	0	0	0	0	1
Missoula	0		0	0	0	1	0	0	0	0	5
Idaho:											
Boise	0		0	5	2	0	2	0	0	3	10
Colorado:											
Denver	0	43	0	79	8	20	0	6	0	88	97
Pueblo	0		0	0	0	2	0	2	0	13	14
New Mexico:											
Albuquerque	0		0	2	2	2	0	2	0	7	9
Utah:											
Salt Lake City	0		0	506	5	4	0	1	0	18	38

City reports for week ended Mar. 3, 1934—Continued

State and city	Diphtheria cases	Influenza Cases	Influenza Deaths	Measles cases	Pneumonia deaths	Scarlet fever cases	Smallpox cases	Tuberculosis deaths	Typhoid fever cases	Whooping cough cases	Deaths, all causes
Nevada:											
Reno	0		0	2	1	1	0	0	0	0	6
Washington:											
Seattle	0		2	1	6	26	4	4	1	73	75
Spokane	0			85	2	3	0	1	0	9	29
Tacoma	0		0	16	4	3	0	0	0	16	33
Oregon:											
Portland	0		1	2	11	11	0	3	0	7	82
Salem	0	3	0	0	0	0	0	0	0	2	
California:											
Los Angeles	20	39	0	48	17	46	0	22	0	52	296
Sacramento	1	4	1	4	5	1	0	4	0	1	38
San Francisco	0	3	1	105	15	17	0	8	1	26	160

State and city	Meningococcus meningitis Cases	Meningococcus meningitis Deaths	Poliomyelitis cases
Massachusetts:			
Boston	2	0	0
New York:			
New York	2	4	1
Pennsylvania:			
Philadelphia	1	0	0
Ohio:			
Cleveland	1	0	0
Illinois:			
Chicago	4	2	0
Michigan:			
Grand Rapids	0	0	1
Minnesota:			
Duluth	1	0	0
Missouri:			
Kansas City	1	0	0
Tennessee:			
Memphis	0	1	0
Alabama:			
Birmingham	0	1	0
Texas:			
Fort Worth	0	1	0
Colorado:			
Denver	0	1	0
Utah:			
Salt Lake City	1	0	0

Lethargic encephalitis.—Cases: Springfield, Mass., 1; Grand Rapids, 1; San Francisco, 1.

Pellagra.—Cases: Miami, 1; Tampa, 1; Memphis, 2; Montgomery, 1; New Orleans, 1; San Francisco, 1.

Typhus fever.—Cases: New York, 1; Galveston, 1.

FOREIGN AND INSULAR

BELGIUM

Deaths during 1932.—During the year 1932, 108,226 deaths occurred in Belgium, giving a rate of 13.18 per 1,000 population. Deaths from certain causes were reported as follows:

Disease	Number of deaths	Deaths per 100,000 population	Disease	Number of deaths	Deaths per 100.000 population
Bronchitis	3,132	38.1	Nephritis	2,456	29.9
Cancer and other malignant tumors	8,267	100.7	Pneumonia	7,910	96.3
Cerebral hemorrhage	7,618	92.8	Puerperal septicemia and puerperal infections	250	3.0
Diarrhea and enteritis (under 2 years)	1,679	20.4	Scarlet fever	150	1.8
Diphtheria	464	5.6	Syphilis	83	1.0
Heart disease	16,438	200.2	Tuberculosis, pulmonary	5,247	63.9
Influenza	3,110	37.9	Tuberculosis, other forms	1,527	18.6
Malaria	10		Typhoid and paratyphoid fever	178	2.2
Measles	477	5.8	Whooping cough	647	7.9

CANADA

Provinces—Communicable diseases—2 weeks ended February 24, 1934.—During the 2 weeks ended February 24, 1934, cases of certain communicable diseases were reported by the Department of Pensions and National Health of Canada, for 8 provinces, as follows:

Disease	Prince Edward Island	Nova Scotia	New Brunswick	Quebec	Ontario	Manitoba	Saskatchewan	British Columbia	Total
Cerebrospinal meningitis				1	1				2
Chicken pox		26	8	223	444	75	82	76	934
Diphtheria		14		32	16	15	13	2	92
Dysentery							3		3
Erysipelas		1		26	6	1	2		36
Influenza	92	20		15	32	55		32	246
Lethargic encephalitis							1		1
Measles		7	2	172	60	273	322	8	844
Mumps					281	10	9	136	436
Pneumonia		5			28		4	12	49
Poliomyelitis		1		3					4
Scarlet fever	1	18	2	164	281	38	9	196	709
Smallpox							1	4	5
Trachoma							1	2	3
Tuberculosis	2	6	1	100	54	7	5	54	229
Typhoid fever			3	69	3		2		77
Undulant fever				1	3		1		5
Whooping cough		7		509	241	74	72	27	930

NOTE.—No report was received from Alberta for the above period.

GREAT BRITAIN

Scotland—Vital statistics—Quarter ended December 31, 1933.—The Registrar General of Scotland has published the following vital statistics for Scotland for the fourth quarter, ended December 31, 1933:

Population, estimated	4,916,000
Births	20,415
Birth rate per 1,000 population	16.5
Deaths	15,883
Death rate per 1,000 population	12.8
Deaths under 1 year	1,612
Deaths under 1 year per 1,000 births	79
Marriages	8,723
Deaths from:	
Bronchitis	785
Broncho-pneumonia	515
Cancer	1,982
Cerebrospinal fever	33
Diabetes	200
Diphtheria	117
Dysentery	3
Erysipelas	68
Heart disease	2,726
Deaths from—Continued.	
Influenza	164
Lethargic encephalitis	20
Measles	2
Nephritis, acute	56
Nephritis, chronic	301
Nephritis, unspecified	111
Paratyphoid fever	4
Pneumonia (lobar)	351
Pneumonia, unspecified	230
Poliomyelitis	5
Puerperal sepsis	62
Scarlet fever	122
Syphilis	16
Tetanus	4
Tuberculosis	870
Typhoid fever	4
Whooping cough	56

Vital statistics—Year 1933.—The following table shows the provisional figures for Scotland for the year 1933:

Births	86,546
Birth rate per 1,000 population	17.6
Deaths	64,848
Death rate per 1,000 population	13.2
Deaths under 1 year	7,019
Deaths under 1 year per 1,000 births	81
Marriages	34,215
Deaths from:	
Bronchitis	3,289
Cancer	7,518
Cerebrospinal fever	220
Cirrhosis of liver	131
Diabetes	711
Deaths from—Continued.	
Diphtheria	356
Heart disease	10,488
Influenza	2,027
Measles	36
Nephritis, acute and chronic	1,749
Pneumonia (all forms)	4,599
Puerperal sepsis	213
Scarlet fever	310
Suicide	523
Tuberculosis	3,910
Typhoid fever	30
Whooping cough	762

INDIA

Vital statistics.—According to the 1931 census of India, the population of that country was 353,837,778, representing an increase of 10.6 percent since the census of 1921. The density of population ranged from 6.5 persons per square mile in the arid regions of Sind and Baluchistan to 814.2 in Cochin State and 935 in Bengal. The average density for the entire country was 195 persons per square mile. The population of British India was 256,859,787 as compared with 81,310,845 for the native States.

The birth rate for 1930 was 33.2 per 1,000 population, and the death rate was 26.1 per 1,000. The infant mortality rate was 180.8 in 1930, as compared with 194.9 in 1920. By far the greater number of deaths among infants under 1 year were said to be due to infantile debility, malformation, and respiratory diseases. Despite the high death rates the excess of births over deaths during the period 1921–31 was 20,000,000.

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

(NOTE.—A table giving current information of the world prevalence of quarantinable diseases appeared in the PUBLIC HEALTH REPORTS for Feb. 23, 1934, pp. 276–288. A similar cumulative table will appear in the PUBLIC HEALTH REPORTS to be issued Mar. 30, 1934, and thereafter, at least for the time being, in the issue published on the last Friday of each month.)

CHOLERA

Philippine Islands.—During the week ended March 10, 1934, cholera was reported in the Philippine Islands as follows: Bohol Province—Calape, 3 cases, 1 death; Clarin, 4 cases, 2 deaths; Inabanga 17 cases, 5 deaths; Loon, 3 deaths; Tagbilaran, 1 case, 1 death; Talibon, 13 cases, 7 deaths; Tubigon, 11 cases, 7 deaths. Oriental Negros Province—Tanjay, 13 cases, 6 deaths.

SMALLPOX

Mexico—Coahuila—Monclova.—A report dated March 3, 1934, states that 8 cases of smallpox have appeared in Monclova, Coahuila, Mexico. One death has been reported.

Palestine.—During the week ended March 3, 1934, 10 cases of smallpox were reported in Palestine.

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