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A METHOD OF CONDUCTING THE 50 PERCENT HEMOLYSIS END POINT COMPLEMENT-FIXATION TEST FOR PARASITIC DISEASES [1]

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Those who have conducted complement-fixation tests for helminthic and protozoan diseases have soon discovered that the complex nature of the antigens renders the tests unreliable, a result mainly due to the anticomplementary effect of the antigen. It is believed that the technique described below will eliminate most of the difficulties encountered previously. This test is similar in nature to the techniques of Wadsworth (*1*), Wadsworth, Maltaner, and Maltaner (*2*), and Kent, Bucantz, and Rein (*3*). The complement titer is determined on the basis of that amount which will give 50-percent hemolysis when compared to the color standard. The test is conducted in a total volume of 1 cc. in which 4 of these 50-percent units are used.

Preparation of the 2-percent cell suspension.—Before the optimal amboceptor titer is determined, it is necessary to prepare a 2-percent suspension of cells from which a color standard is made in order to determine the end point for 50-percent hemolysis. Sheep cells are collected by using sodium citrate or by defibrination. If it is desired to hold the cells for a period over 3 days, a portion should be removed from the original collecting container and washed four times with physiologic saline solution. If it is necessary to perform titrations with the same cell suspension for a period of several weeks, it will be

[1] From the Zoology Laboratory, National Institute of Health.

found advisable to use Alsever and Ainslie's (*4*) solution or Bucantz, Rein, and Kent's (*5*) modification of this solution for the preservation of cells to reduce fragility and consequent hemolysis. After the final washing has been completed, a 15-cc. graduated, conical centrifuge tube is filled with washed cells which are centrifuged at 2,000 r. p. m. for 10 minutes. The quantity of packed cells is measured and the volume occupied by the cells is multiplied by a factor of 50 to make a 2-percent suspension. This will give the total volume which must be made up with saline. Thus, if the volume of packed cells is 1.5 cc., then $1.5 \times 50 = 75$ cc. Therefore, the contents of the tube can be poured into a graduated cylinder and the centrifuge tube washed with saline. The washings are added to the graduated cylinder. Saline is then added until a volume of 75 cc. is reached.

Preparation of the hemoglobin standard.—After the 2-percent cell suspension is made, 10 cc. of it is pipetted into a graduated centrifuge tube and packed at 2,000 r. p. m. for 10 minutes. Without disturbing the cells, almost all of the supernatant fluid is withdrawn by means of a capillary pipette. Distilled water is added up to the 9.50-cc. mark; the cells laked completely by shaking; and 0.50 cc. of a stock buffer solution or the same volume of a 17-percent saline solution added to restore tonicity.

Stock buffer solution

NaCl	17.00 gm.
Na_2HPO_4	1.13 gm.
KH_2PO_4	0.27 gm.
Distilled water to make	100.00 cc.

This stock solution may be employed in place of saline as a diluent for all the reagents if 1 part of the stock solution is added to 20 parts of distilled water.

After preparing the standard hemoglobin solution, the color standard is prepared from it in the same diameter tubes which are to be used in the test proper. Since the total volume in the test is 1 cc., all the titrations are conducted on the same volume and the measurement of the reagents given in all the tables henceforth will be in tenths of a cubic centimeter.

TABLE 1.—*Preparation of color standard*

Percent of hemolysis	0	10	20	30	40	50	60	70	80	90	100
Saline	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
Standard hemoglobin	0	.02	.04	.06	.08	.10	.12	.14	.16	.18	.20
2 percent red cells	.20	.18	.16	.14	.12	.10	.08	.06	.04	.02	0

The tubes are shaken and labeled according to the degree of hemolysis and then centrifuged at 2,000 r. p. m. for 10 minutes.

Titration of amboceptor.—It is advisable to begin the titration of amboceptor with a 1:2,000 dilution; if the pooled amboceptor does

not attain this titer it is not satisfactory and the animals should be given further immunization. To prepare the sensitized cells for this titration, the amboceptor dilution desired is made up in 10-cc. amounts and an equal volume of the 2-percent cell suspension is added. The sensitized cells must be prepared 15 minutes before using them in the test. A 1:100 dilution of complement is prepared from the pooled serum of several guinea pigs.

The tubes for the initial titration of amboceptor are arranged according to table 2. In this preliminary titration of amboceptor, the complement titration is performed at the same time.

TABLE 2.—*Amboceptor titration*

Tube No.	1	2	3	4	5	6	7	8	9	10
Saline	0.55	0.50	0.45	0.40	0.35	0.30	0.25	0.20	0.15	0.10
Complement	.05	.10	.15	.20	.25	.30	.35	.40	.45	.50
Sensitized cells	.40	.40	.40	.40	.40	.40	.40	.40	.40	.40

The above arrangement is made for each dilution of amboceptor to be tested. The titration is then placed in a 37° C. water bath for 30 minutes after which it is removed and the tubes centrifuged at 2,000 r. p. m. for 10 minutes. Each tube is then compared with the color standard tubes and the results in percent of hemolysis are recorded according to the plan given below:

TABLE 3.—*Reading of amboceptor titration*

Tube No.	1	2	3	4	5	6	7	8	9	10
Amboceptor dilution										
1:7,000	0	0	0	0	5	10	20	25	35	45
1:6,500	0	0	0	8	20	30	40	55	65	68
1:6,000	0	0	3	10	25	35	50	60	65	70
1:5,500	0	0	5	15	35	45	55	70	80	85
1:5,000	0	0	8	30	50	60	80	85	90	95
1:4,500	0	0	20	40	60	70	85	88	95	98
1:4,000	0	0	25	50	75	80	85	90	95	100
1:3,500	0	10	30	60	80	85	95	100	100	100
1:3,000	0	30	55	80	90	95	98	100	100	100
1:2,500	0	35	60	85	93	98	100	100	100	100
1:2,000	2	37	65	90	95	100	100	100	100	100

The optimal dilution of amboceptor as defined by Wadsworth (*1*) is that dilution beyond which further increase in the concentration of amboceptor does not appreciably change the quantity of complement required for 50 percent hemolysis. Therefore, from the above titration it is apparent that the optimal dilution of amboceptor is 1:3,000. This concentration is used henceforth for the daily titration of complement and for the sensitization of red cells for the test proper. The above titration served a dual purpose in this instance in that both the complement and amboceptor titration were conducted at the same time.

Titration of complement.—The complement is titrated daily with the optimal dilution of amboceptor as indicated in the above table. Fresh, pooled serum obtained from several guinea pigs is diluted 1:100 and the titration is made according to table 1. In this titration only the optimal dilution of amboceptor is used. After incubation and centrifugation, each tube in the test is compared to the 50 percent hemolysis tube in the color standard. The color standard must be prepared fresh daily with a portion of the cells from the same lot which is to be used in the test proper. If the color standard tube containing the 50 percent hemolysis matches exactly tube No. 3 of the titration containing 0.15 cc. of 1:100 complement, tube No. 3 is regarded as 1 unit of complement. If the 50 percent color standard falls between 2 tubes, it is necessary to interpolate to obtain the 1 unit of complement. Since 4 units of complement are required in each 0.20 cc., the dilution of complement is made according to the following formula: $4\times0.15=0.60$ cc. of 1:100 complement. Consequently $\frac{0.60}{100}=\frac{0.20}{x}=33.3$. Therefore, 0.20 cc. of a 1:33.3 dilution of complement will be required for the test. This dilution is also employed in the controls in order to determine quantitatively the amount of complement which may deteriorate in the presence of serum, antigen, and saline alone.

METHOD OF CONDUCTING THE DIAGNOSTIC TEST

The serum submitted for the complement-fixation tests should be inactivated for 30 minutes at 56° C. just prior to being used. If the serum was inactivated the day before, it is advisable to reheat the serum at the above-mentioned temperature for about 5 minutes before using it. Two dilutions of serum are employed in the test, 1:5 and 1:10 dilutions. The test is arranged and the reagents added in the order given in table 4. The optimal dilution of the specific antigen should be employed.

TABLE 4.—*The test proper*

Tube No.	1	2	3	4	5	6	7	8	9	10	11
Reagents											
Saline			0.30	0.25	0.20	0.30	0.25	0.20	0.30	0.25	0.20
Serum 1:5	0.20		.20	.20	.20						
Serum 1:10		0.20				.20	.20	.20			
Complement (4 units)	.20	.20	.10	.15	.20	.10	.15	.20	.10	.15	.20
Antigen	.20	.20							.20	.20	.20

The tubes are placed in the refrigerator overnight (15–18 hours) at 5° C. The next day 0.40 cc. of sheep cells which have been sensitized 15 minutes previously are added to each tube and the tubes placed

in a 37° C. water bath for 30 minutes. They are then removed and read.

INTERPRETATION OF RESULTS

In the usual complement-fixation technique the amount of complement which will deteriorate overnight in the presence of serum or antigen is not taken into consideration. By the procedure described in this paper, it can be seen that the amount of deterioration which might occur with any given serum sample is taken into consideration in evaluating the final results of the test. For the purpose of convenience this deteriorating factor will be called the binding power of the serum or of the antigen. In table 4, tubes No. 3, 4, and 5 contain, respectively, 2, 3, and 4 50-percent units of complement to determine the binding power of the 1:5 serum dilution. Tubes No. 6, 7, and 8 containing, respectively, 2, 3, and 4 50-percent units of complement should give the amount of complement bound in the 1:10 serum dilution. Tubes No. 9, 10, and 11 should show the quantity of complement bound by the antigen employed in the test. Thus, it can be seen that the binding power is determined for both dilutions of serum and the antigen employed in the test if any binding should exist. It is necessary to conduct binding powers on every single serum used in the test, but it is only necessary to conduct one set of binding powers for the antigen for the particular day that the antigen is used.

Tubes containing 30, 50, 70, and 100 percent cells, respectively, of the color standard are removed and shaken thoroughly. Any tube in the test matching the tube containing 30 percent cells or more is regarded as a positive, and any tube showing less than 30 percent fixation is regarded as negative. The procedure given in this article is intended for general laboratory use since most laboratories do not usually possess a spectrophotometer for refined measurements.

The advantage of the present method of conducting the complement fixation test may be readily illustrated by a few examples. If a serum should give the following fixation results, it is apparent from tubes No. 3, 4, and 5 that the serum itself will bind 3 units of the complement.

Tube No.	1	2	3	4	5	6	7	8	9	10	11
1:5 serum	++++		++++	+++	−						
1:10 serum		+				++++	+	−			
Antigen									−	−	−

Yet, with the usual methods, tube No. 5 would be the control tube and the test would have been regarded as satisfactory. However, the second serum dilution 1:10 also has some binding as disclosed by tube

No. 7. This serum is regarded as a negative when the present method of complement fixation is employed. If the serum showing the binding displayed in the above example had any antibodies present the titer would have been much higher.

At times, it has been found that antigen will begin to deteriorate and tests are made to guard against this, as shown by the following example:

Tube No.	1	2	3	4	5	6	7	8	9	10	11
1:5 serum	++++		−	−	−						
1:10 serum		++++				−	−	−			
Antigen									++++	+++	−

In this case, it is apparent that the antigen, as disclosed by tubes No. 9 and 10, binds at least 3 units of the complement by itself. Again tube No. 11, which is the usual control in other tests, is negative and the antigen would have been regarded as satisfactory. In this instance, it is necessary to repeat the test with a new and satisfactory antigen.

In the following example the test is regarded as positive:

Tube No.	1	2	3	4	5	6	7	8	9	10	11
1:5 serum	++++		++++	++	±						
1:10 serum		++++				+++	±	−			
Antigen									−	−	−

The test is regarded as positive because the serum gave good fixation in the 1:10 dilution and in this dilution the anticomplementary effect of the serum is practically removed. The 1:5 dilution could not be read safely because of the strong anticomplementary tendencies.

REFERENCES

(*1*) Wadsworth, A. B.: Standard methods of the Division of Laboratories and Research of the New York State Department of Health. Baltimore, Williams and Wilkins, 1927.

(*2*) Wadsworth, Augustus; Maltaner, Frank; and Maltaner, Elizabeth: Quantitative studies of the complement-fixation reaction with syphilitic serum and tissue extract; technic of the practical quantitative test. J. Immunol., **35**: 217–234 (September 1938).

(*3*) Kent, J. F.; Bucantz, S. C.; and Rein, C. R.: Studies in complement-fixation. I. Spectrophotometric titration of complement, construction of graph for direct determination of the 50 percent hemolytic unit. J. Immunol. (In press.)

(*4*) Alsever, John B., and Ainslie, Robert B.: A new method for the preparation of dilute blood plasma and the operation of a complete transfusion service. New York State J. Med., **41**: 126–135 (January 1941).

(*5*) Bucantz, S. C.; Rein, C. R.; and Kent, J. F.: Studies in complement-fixation. II. Preservation of sheep's blood in citrate-dextrose mixtures (modification Alsever's solution) for use in the complement-fixation reaction. J. Lab. & Clin. Med. (In press).

STREPTOMYCIN IN EXPERIMENTAL PLAGUE [1]

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Recently Schatz, Bugie, and Waksman (*1*) isolated from an organism resembling *Actinomyces griseus* a substance, streptomycin, which is more active against several organisms than is streptothrycin. Its toxicity is low (LD 0–35 mg.; LD 100–135 mg. per 20-gm. mouse with a preparation containing 30,000 units per gram) (*2*). In view of its possibilities as a therapeutic agent in human plague the following tests were made.

MATERIALS AND METHODS

The material [2] used in these experiments contained approximately 200,000 units of streptomycin per gram.

The toxicity of this preparation was not properly tested because of a shortage of material. However, one 14-gm. mouse given 20 mg. as a 5-percent solution intraperitoneally died in 20 minutes while one 15-gm. mouse receiving 10 mg. survived. A 16-gm. mouse given subcutaneously 40 mg. as a 5-percent solution in distilled water survived.

Serial dilutions of the material were made in a liquid medium consisting of tryptose 1.5 percent, sodium chloride 0.5 percent, and glucose 0.1 percent. Dilutions were threefold and ran from 1/4,000 to 1/972,000. All tubes were inoculated with a broth culture of *Pasteurella pestis* and incubated 3 days at 37° C. Growth or inhibition was determined by turbidity.

In animal experiments, mice weighing about 18 gm. were inoculated subcutaneously in the groin with 0.2 cc. of a suspension of *P. pestis* in 1-percent peptone water. The inoculum contained up to 6,000 organisms as determined by plating methods. The streptomycin was inoculated intraperitoneally as a 1-percent solution at various times and in varying amounts. Controls were given the same volume of normal saline at the time the test animals received the drug.

In two tests (tables 4 and 5), treatment was started 3 and 2 days, respectively, following inoculation with live organisms. Visible swelling at the site of inoculation was considered one of the signs of infection; therefore at the time treatment was started animals not showing visible swelling in the groin (and those that had died) were excluded from the experiment. The remainder were treated. Sulfadiazine was also used for purposes of comparison; results are shown in table 5.

[1] From The States Relations Division (Plague Laboratory, San Francisco, Calif.). This paper was received for publication Oct. 16, 1944, and scheduled for publication in PUBLIC HEALTH REPORTS in the issue of Nov. 10, 1944. Because of the subject matter the paper was withheld from publication at that time.

[2] Streptomycin was supplied through the courtesy of Merck & Co., Inc., Rahway, N. J.

At the end of 14 days from the time of inoculation, all surviving animals were killed, autopsied, and the spleens cultured (circumstances prevented spleen culture in the last experiment). Animals dying during the test were autopsied, but spleen cultures were not made, as decomposition made the isolation of pure cultures difficult. Spleen smears were made on all animals in which the post-mortem appearance was not typically that of plague in all its details. When typical bipolar organisms were found the spleen smears were considered positive. In diagnosing plague by post-mortem appearance, the following signs were looked for: Local lesions; enlarged lymph nodes; injected skin; soft, dark, enlarged spleen; lesions in the liver, lungs, and spleen. Spleen cultures were made by rubbing the cut surface of the spleen on the surface of a tryptose-glucose agar plate. A number of the positive cultures were checked by transfer of the typical colonies to tubes where fermentation of the various sugars was checked.

RESULTS

Streptomycin (both unheated and heated) inhibits the growth of *P. pestis* in broth, as shown in the accompanying table.

TABLE 1

	Dilution of drug which—	
	Inhibits growth of *P. pestis*	Allows growth of *P. pestis*
Streptomycin unheated	1/160,000	1/320,000
Streptomycin heated 56° C. 30 minutes	1/160,000	1/320,000
Streptomycin heated 100° C. 10 minutes	1/160,000	1/320,000

To test the prophylactic action of the drug mice were inoculated with plague subcutaneously, and varying amounts of streptomycin given intraperitoneally ½ hour before and 17 and 24 hours after challenge. It seemed justifiable to consider mice dead 14 days following plague inoculation to have died from plague. However, autopsies were done with the following results. All of the animals which died showed evidence of plague on the basis of either gross pathology, or spleen smears, or both. Two of the four controls killed at the end of 14 days had local lesions, and one had a bubo on the side opposite to the site of injection. One of the mice receiving dosage of 1 mg. and one receiving 0.5 mg. had enlarged inguinal nodes. The remainder were essentially normal. Spleens of all of the mice killed were negative on culture. Results of the test are tabulated in table 2.

TABLE 2

Streptomycin dosage (milligrams per dose)	Mortality		
	Number of animals	Number dead in 14 days	Percent dead in 14 days
0.0 (controls)	15	11	74
2.0	10	0	0
1.0	5	1	20
0.5	5	4	80

The above test was repeated, but the drug was given ½ hour before and 24 hours after infection (making two instead of three doses). In addition, in one group treatment was not started until 2 days following infection. Results found at autopsy were as follows. All animals which died showed evidence of plague on the basis of either gross pathology, or spleen smears, or both. Surviving animals were killed on the fourteenth day. Three of the controls showed enlarged inguinal glands. One mouse receiving 1 mg. and one receiving 0.5 mg. had local lesions. One mouse given 0.5 mg. had both local and splenic lesions. One mouse given delayed treatment had local lesions. Spleen cultures of one mouse receiving 2 mg. and one receiving 0.5 mg. were positive for plague. All other spleen cultures from killed mice were negative. Results of the tests are shown below.

TABLE 3

Streptomycin dosage (milligrams per dose)	Mortality			Average survival in days
	Number of animals	Number dead in 14 days	Percent dead in 14 days	
0.0 (controls)	10	7	70	7.6
2.0	10	0	0	----------
1.0	5	1	20	----------
0.5	5	3	60	----------
2.0 (first dose 48 hours after challenge)	5	4	80	9.7

In order to determine the effectiveness of streptomycin after the infection had developed, 30 mice were challenged with live organisms. At 72 hours, 2 were dead and 3 showed no swelling at the site of inoculation; these were discarded. The remainder were treated. Two doses were given with a 24-hour interval between. All animals that died showed evidence of plague on the basis of gross pathology or spleen smears, or both. All surviving animals killed on the fourteenth day had enlarged nodes and one had a local lesion; the cultures from the spleens were negative. Results are shown in table 4.

The test appeared a bit drastic as several of the mice had died before treatment had started. In the following test, treatment was started

TABLE 4

Streptomycin dosage (milligrams per dose)	Mortality			Average survival in days
	Number of animals	Number dead in 14 days	Percent dead in 14 days	
0.0 (controls)	9	9	100	6.0
4.0	7	7	100	9.1
2.0	9	6	66	7.8

48 hours after infection and a dose given daily for 6 days. In addition, a control was run with sodium sulfadiazine in the same dosage except for the first dose, which was 5 mg. rather than 2 mg. Thirty-six mice were given live organisms, and 48 hours later six were discarded as they showed no inguinal swelling. The remainder were treated. All mice which died showed evidence of plague on the basis of gross pathology or spleen smears, or both. All mice killed on the fourteenth day showed swollen inguinal glands. Spleen smears were negative except for one streptomycin-treated mouse which showed an occasional organism. Results of this series are shown in table 5.

TABLE 5

Dosage in milligrams and drug	Mortality			Average survival in days
	Number of animals	Number dead in 14 days	Percent dead in 14 days	
0.0 (controls)	9	8	88	6.1
2.0 sodium sulfadiazine	11	7	63	7.4
2.0 streptomycin	10	1	10	12.0

SUMMARY

Streptomycin is thermostable. It is inhibitory to *P. pestis* in broth in a dilution of approximately 1/160,000 (using a 200,000 unit per gram preparation).

When 2 mg. (400 units) of streptomycin were given before and 2 mg. 24 hours after challenge, 10 mice survived for 14 days a dose of plague organisms which killed 70 percent of the controls.

When treatment (2 mg. per day) was started 2 days following inoculation and continued for 6 days, 9 of 10 mice survived for 14 days. When sodium sulfadiazine was used under the same circumstances, 4 of 11 survived; 8 of 9 controls died.

REFERENCES

(*1*) Schatz, A.; Bugie, E.; and Waksman, S. A.: Streptomycin, a substance exhibiting antibiotic activity against gram positive and gram negative bacteria. Proc. Soc. Exper. Biol. & Med., **55**:66 (1944).

(*2*) Jones, D.; Metzgar, H. J.; Schatz, A.; and Waksman, S. A.: Control of gram negative bacteria in experimental animals by streptomycin. Science, **100**:103 (Aug. 4, 1944).

SEQUESTRATION OF CALCIUM AND MAGNESIUM IN THE PRESENCE OF ALKALINE DETERGENTS [1]

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Uncontrolled water hardness is detrimental to cleaning processes in which alkaline compounds or soap are used at elevated temperatures. Hardness in natural waters is due to the solution of calcium and magnesium salts. These salts vary in concentration in supplies throughout the United States from 5 p. p. m. to about 500 p. p. m. expressed as calcium carbonate. With few exceptions the alkalies used in detergents form insoluble compounds with calcium and magnesium and both are precipitated when water is heated.

Water hardness interferes with cleaning processes in two ways, by reducing the active detergent content of the solution through precipitation, necessitating the use of greater amounts of detergent, and by producing films and water spots on the cleaned utensil.

The solubilities of calcium salts produced by the usual alkalies employed in detergents are very low, ranging from 20 p. p. m. to 90 p. p. m., while those of magnesium are somewhat higher, being from 100 p. p. m. to 200 p. p. m. Certain synthetic detergents or wetting agents used in commercial detergents also react with the hardness of the water to give precipitates of calcium and magnesium salts.

The elimination of these troublesome factors may be brought about in two ways, by complete softening of the water prior to use, which in most cases is impossible or at least impractical, or by using certain complex polyphosphates which have the ability to sequester the hardness of the water, thus inhibiting the formation of insoluble calcium and magnesium salts.

The term sequestration implies that property of a compound or mixture which enables it to form soluble salts with the calcium and magnesium present in natural waters, thus preventing their precipitation by heat or by other substances present.

There are three compounds commonly employed in the manufacture of detergents which possess the property of sequestration, namely, sodium hexametaphosphate, tetrasodium pyrophosphate, and sodium tetraphosphate.

Sodium hexametaphosphate and sodium tetraphosphate must be used in a mixture containing other alkaline materials, due to their lack of active alkalinity and practically neutral pH values. Tetrasodium pyrophosphate, on the other hand, has a considerably higher active alkalinity and higher pH value and may be used without fortification with other alkalies for some cleaning operations.

[1] From the Sanitary Engineering Division, Water and Sanitation Investigations, Cincinnati, Ohio.

Tests were run on various mixtures of these three sequestering agents with other commonly employed alkalies, namely, sodium hydroxide, sodium carbonate, trisodium phosphate, sodium metasilicate and sodium sesquisilicate, to determine how much hardness could be sequestered. These tests were run for both calcium and magnesium at temperatures of 140° F. and 200° F.

EXPERIMENTAL METHOD

A total concentration of 0.3 percent dissolved material including both the sequestering agent and the alkaline detergent was chosen as the average concentration of detergent recommended for use in food and dairy sanitizing processes. Various ratios of the alkalies and each of the sequestering agents were made up with a total concentration of 0.3 percent and the solutions were heated to 140° or 200° F., as desired for the particular test. These temperatures were selected because they are representative and desirable temperatures for washing and rinsing in mechanical dishwashing. Portions of 100 ml. of the mixed detergent solutions at the temperatures mentioned were titrated by adding standard calcium and magnesium chloride solutions from a burette until precipitation started to take place. The titration was made in a 500-ml. Erlenmeyer flask against a dull black background with indirect illumination through the test liquid. From these titrations calculations were made of the quantities of calcium and magnesium expressed in parts per million of calcium carbonate tolerated by the solution at the given temperature before precipitation occurred.

EXPERIMENTAL RESULTS

The study showed that the presence of other ions and temperature affects the sequestering ability of all three of the agents used. In general, it is easier to sequester both calcium and magnesium at lower temperatures. The data obtained have been plotted in a series of curves for each sequestering agent (figs. 1, 2, 3), and table 1 shows the sequestering values obtained with equal quantities of the phosphates and alkaline detergents at the two temperatures. These data show that in general it is harder to prevent the precipitation of calcium hardness than magnesium hardness by these agents. Also, as natural waters generally have hardness ratios of two parts calcium to one part magnesium, it may be stated that if a particular detergent mixture adequately controls the calcium hardness of the water, precipitation of the magnesium will also be prevented.

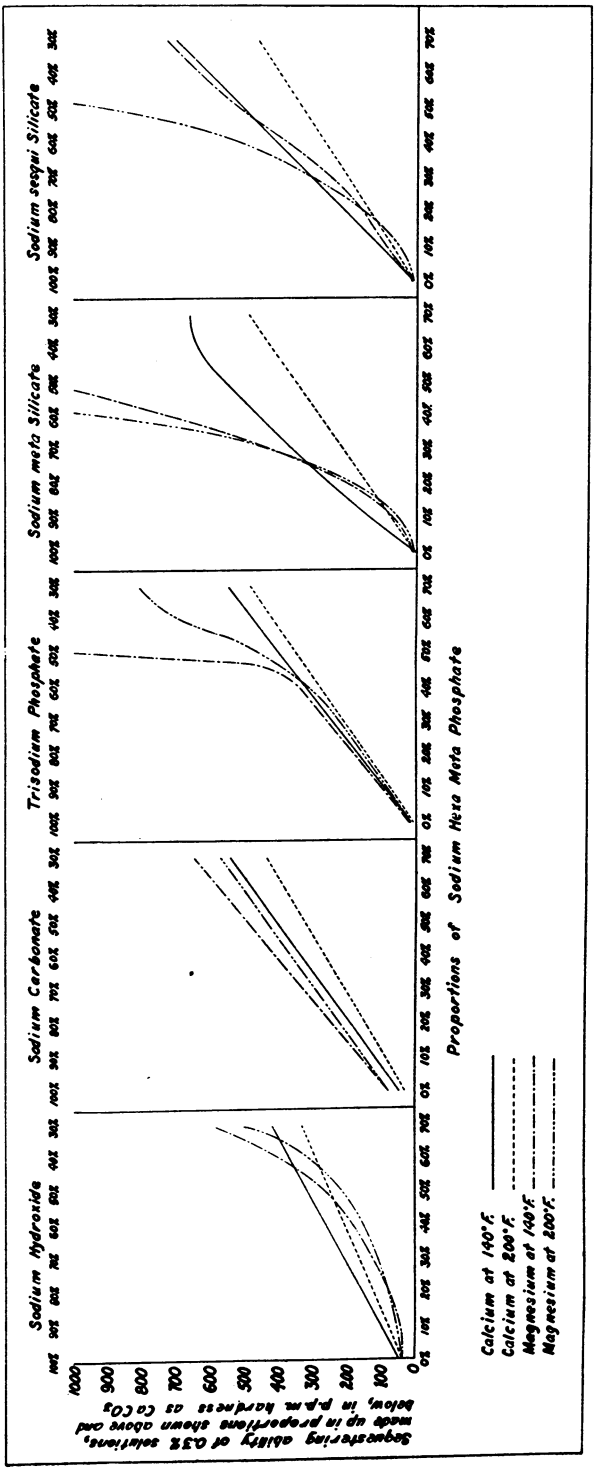
Sodium Hydroxide
Sodium Carbonate
Trisodium Phosphate
Sodium meta Silicate
Sodium sesqui Silicate
Sequestering ability of 0.3% solutions, made up in proportions shown above and below, in p.p.m. hardness as $CaCO_3$
1000
900
800
700
600
500
400
300
200
100
0
Proportions of Sodium Hexa Meta Phosphate
Calcium at 140°F.
Calcium at 200°F.
Magnesium at 140°F.
Magnesium at 200°F.

FIGURE 1.

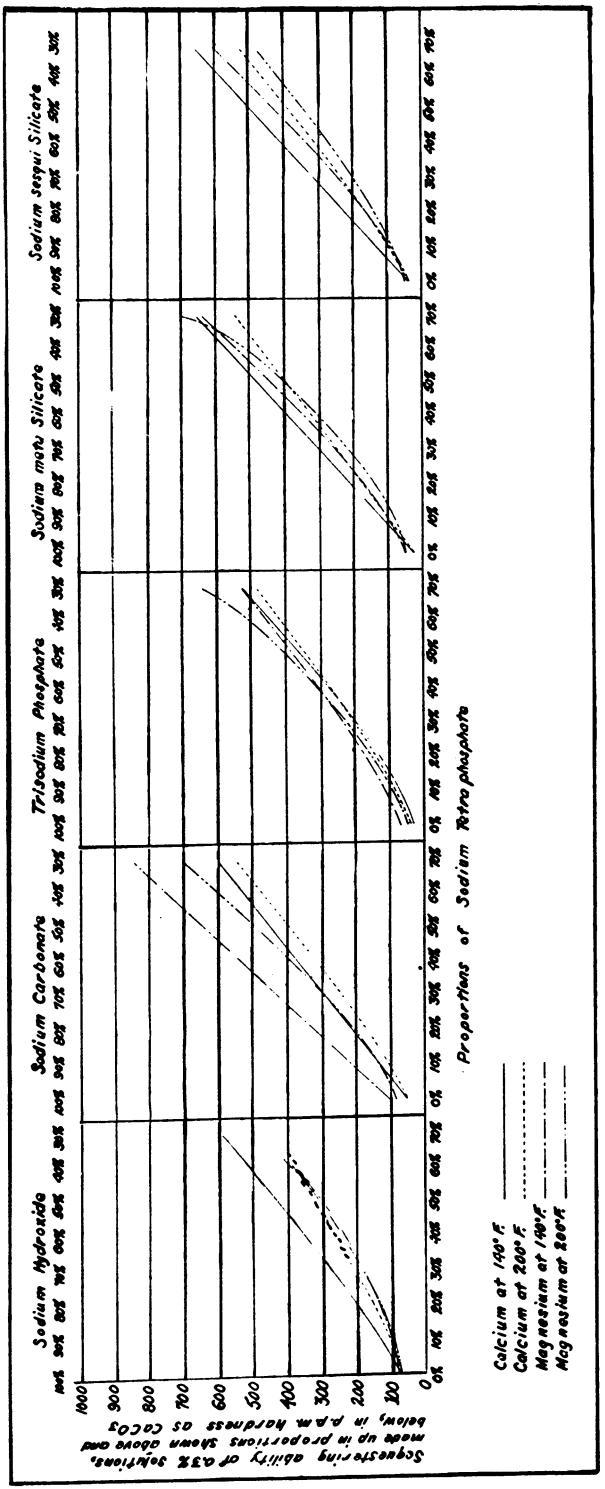
Sequestering ability of 0.3% solutions, made up in proportions shown above and below, in P.P.M. hardness as CaCO3
1000
900
800
700
600
500
400
300
200
100
0
Sodium Hydroxide
Sodium Carbonate
Trisodium Phosphate
Sodium meta Silicate
Sodium Sesqui Silicate
Properties of Sodium Tetra phosphate
Calcium at 140° F.
Calcium at 200° F.
Magnesium at 140° F.
Magnesium at 200° F.

FIGURE 2.

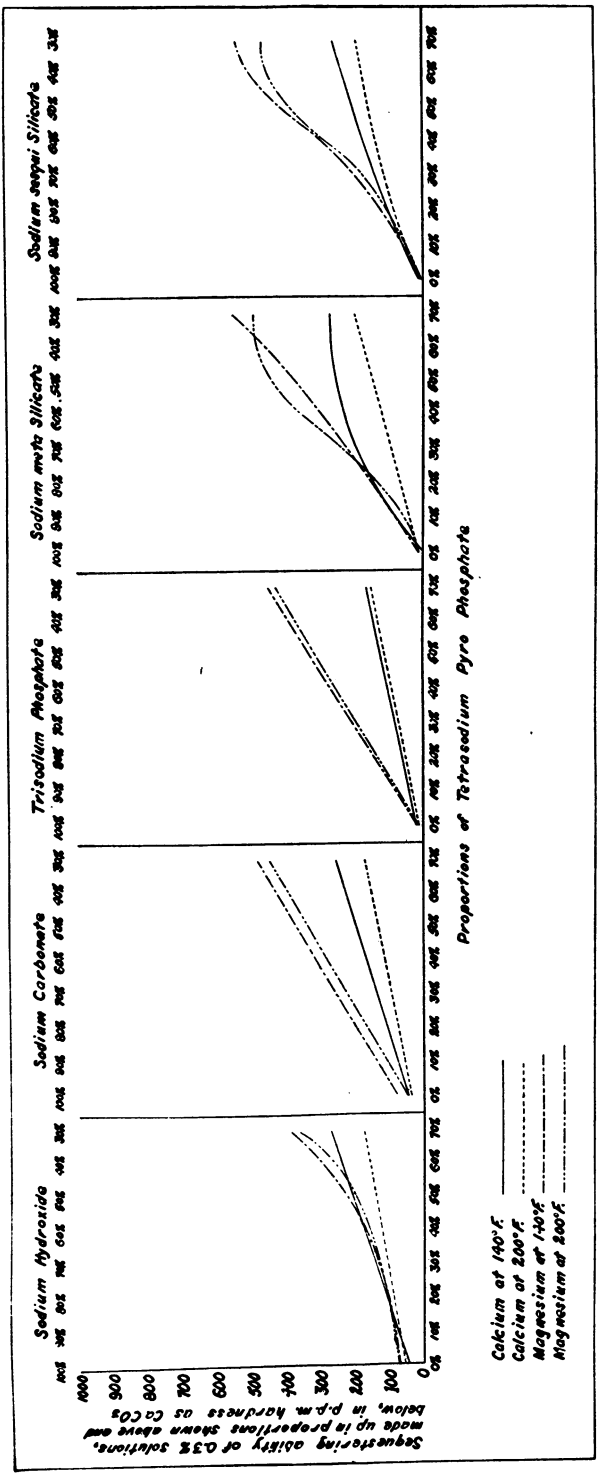
Sodium Hydroxide
Sodium Carbonate
Trisodium Phosphate
Sodium meta Silicate
Sodium Ortho Silicate
Sequestering ability of 0.3% solutions, made up in proportions shown above and below, in p.p.m. hardness as CaCO3
1000
900
800
700
600
500
400
300
200
100
0
Calcium at 140°F.
Calcium at 200°F.
Magnesium at 140°F.
Magnesium at 200°F.
Proportions of Tetrasodium Pyro Phosphate

FIGURE 3.

Sodium hexametaphosphate [2] and sodium tetraphosphate [3] display almost equal sequestering ability for calcium. Sodium hexametaphosphate, in the presence of trisodium phosphate and the silicates, has somewhat better magnesium-sequestering ability than does sodium tetraphosphate. The latter phosphate has approximately the same degree of sequestering ability for both calcium and magnesium.

Of the three phosphates studied, tetrasodium pyrophosphate displays the poorest sequestering ability for calcium, its reactions with magnesium being comparable to those of sodium tetraphosphate.

The presence of other alkaline salts is a determining factor in the ease with which the hardness is controlled. Where sodium hexametaphosphate is used as the sequestering agent, the order in which sequestering ability increases for calcium in the presence of the five alkalies studied is as follows: Sodium hydroxide, sodium carbonate, trisodium phosphate, sodium sesquisilicate, and sodium metasilicate.

In the case of sodium tetraphosphate this order becomes: Trisodium phosphate, sodium hydroxide, sodium carbonate, and the silicates, both compounds having the same effect.

With tetrasodium pyrophosphate, increased sequestering ability occurs as follows: Trisodium phosphate, sodium carbonate, sodium hydroxide, sodium sesquisilicate, and sodium metasilicate. The order of sequestering ability can be checked easily by reference to table 1, which shows the sequestration values in 0.3-percent solutions

TABLE 1.—*Sequestering values for calcium and magnesium in parts per million of $CaCO_3$ of 0.3-percent solutions containing equal quantities of the stated phosphates and alkaline detergents at 140° and 200° F.*

Sequestration:	Polyphosphates	Alkalies				
		NaOH	Na_2CO_3	Na_3PO_4	Metasilicate	Sesquisilicate
Calcium at 140° F	$(NaPO_3)_6$	310	400	400	550	500
	$Na_6P_4O_{13}$	420	440	350	470	490
	$Na_4P_2O_7$	200	190	120	250	200
Calcium at 200° F	$(NaPO_3)_6$	250	320	350	350	330
	$Na_6P_4O_{13}$	330	380	340	390	380
	$Na_4P_2O_7$	140	130	110	140	150
Magnesium at 140° F	$(NaPO_3)_6$	260	490	840	1,000	510
	$Na_6P_4O_{13}$	290	630	370	430	420
	$Na_4P_2O_7$	220	360	320	360	400
Magnesium at 200° F	$(NaPO_3)_6$	210	430	450	>1,000	880
	$Na_6P_4O_{13}$	300	480	390	390	340
	$Na_4P_2O_7$	190	330	300	430	380

containing equal quantities of the phosphates and alkaline detergents at the elevated temperatures of the study.

[2] Product of Calgon, Inc., Pittsburgh, Pa.

[3] Product of Rumford Chemical Works, Rumford, R. I.

DISCUSSION

Dishwashing experiments conducted along with these tests, in which film build-up was studied, indicated that a large portion of film deposited on glassware forms during rinsing. If a detergent precipitates the water hardness instead of sequestering it, an adequate amount of detergent may be added to hard water to soften it by precipitation and still a good job of cleaning may result.

During the rinsing operation, however, the film of detergent remaining on the utensil at the time the rinse water comes into contact with it precipitates the hardness of the rinse water. This precipitation takes place in intimate contact with the utensil and adheres to the surface producing the hardness film.

From these indications it seems of importance to have an adequate quantity of sequestering agent incorporated in the detergent, to prevent precipitation at the start of the rinse. In this way the alkaline film may be rinsed from the utensil before a hardness film has a chance to form.

SUMMARY

The effectiveness of three sequestering agents in preventing the formation of hardness films on utensils during washing operations in solutions of alkaline detergents was studied. The sequestering agents included were sodium hexametaphosphate, tetrasodium pyrophosphate and sodium tetraphosphate. The alkaline detergents were sodium hydroxide, sodium carbonate, trisodium phosphate, sodium metasilicate, and sodium sesquisilicate. Experiments with various ratios of sequestering agent and alkaline detergent in 0.3-percent solutions at 140° and 200° F. showed that in general the sequestering ability was reduced at the higher temperature and that it was affected by the presence of other ions. The sequestration data obtained have been presented in a series of curves. In general, it was found easier to sequester magnesium than calcium. The effectiveness of sodium hexametaphosphate and sodium tetraphosphate in respect to calcium sequestration is about equal, while tetrasodium pyrophosphate is somewhat less effective. Tests in actual washing procedure indicated that precipitation of the hardness during the rinse is very critical in the formation of films on cleaned utensils. This makes essential the use of large proportions of these sequestering agents in detergent mixtures in order to prevent precipitation of water hardness on the utensil in the rinse.

INJURY RESULTING FROM USE OF AEROSOL BOMB [1]

By DWIGHT F. METZLER, *Passed Assistant Sanitary Engineer (R)*

The failure of an aerosol bomb, of the type used by the Army, resulted in a severe hand injury to the person using it. When the needle valve was opened three-quarters turn, it was blown out, allowing the mixture of pyrethrum, DDT, and Freon to escape. The operator placed his hand over the opening in an attempt to stop the flow, and rushed the bomb out of the dwelling. When he dropped it his hand was frozen in the shape in which it had been cupped over the bomb.

Examination of the hand showed injuries equivalent to a third-degree burn. An analysis of the blood indicated no toxic effect from the DDT. The appearance of the bomb revealed that in manufacture the drill had not been properly centered, and that no threads had been cut along one side of the sleeve housing the valve.

[1] From Federal Public Housing Authority, Region III, Chicago, Illinois.

DEATHS DURING WEEK ENDED MAR. 16, 1946

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

	Week ended Mar. 16, 1946	Corresponding week, 1945
Data for 92 large cities of the United States:		
Total deaths	9,231	9,573
Average for 3 prior years	9,662	
Total deaths, first 11 weeks of year	113,120	107,039
Deaths under 1 year of age	592	662
Average for 3 prior years	683	
Deaths under 1 year of age, first 11 weeks of year	6,650	6,989
Data from industrial insurance companies:		
Policies in force	67,185,397	67,133,456
Number of death claims	15,229	15,439
Death claims per 1,000 policies in force, annual rate	11.8	12.0
Death claims per 1,000 policies, first 11 weeks of year, annual rate	11.4	10.9

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 MARCH 23, 1946

Summary

The incidence of diphtheria increased during the week. The total of 368 cases is more than reported for any of the past 4 weeks and more than for the corresponding week of any other year since 1939. An aggregate of 263 cases occurred in the 13 States reporting currently more than 8 cases each, of which the 9 States showing increases are as follows (last week's figures in parentheses): New York 14 (9), Pennsylvania 21 (16), Indiana 14 (6), Illinois 54 (34), Maryland 13 (10), Kentucky 10 (4), Mississippi 13 (5), Louisiana 16 (7), California 25 (24). The total for the year to date is 4,611. The largest number for the corresponding period of the past 5 years, 3,814, occurred in 1942. In 1940 the figure for the period was 4,668.

A total of 34,300 cases of measles was reported for the current week, as compared with 29,812 last week and a 5-year median of 24,632. Of the current total, 19,055 cases, or about 56 percent, occurred in the Middle Atlantic and East North Central areas. The cumulative total is 186,541, as compared with a 5-year median of 184,225.

The total of 166 cases of meningococcus meningitis, as compared with 186 last week and a 5-year median of 225, is the smallest number reported so far this year. The cumulative total, 2,399, is less than reported for any corresponding period since 1942.

Of a total of 28 cases of poliomyelitis (the same number as reported for the corresponding week last year), as compared with 23 last week and 24 for the 5-year median, no State reported more than 2 cases except New York (5) and California (4). The total to date is 494 cases, as compared with 453 for the same period last year and a 5-year median of 311.

Of the total of 14 cases of smallpox reported for the week, 7 occurred in Washington State. A later report states that, up to March 26, 15 cases of the disease have occurred in Seattle and King County, the first case following exposure, on February 5, to a case in a soldier returned from the Orient. Up to March 27, 7 cases had been reported in San Francisco.

Deaths recorded for the week in 93 large cities of the United States totaled 9,569, as compared with 9,267 last week, 9,640 and 9,605 for the corresponding weeks, respectively, of 1945 and 1944, and a 3-year (1943–45) average of 9,747. The total for the year to date is 123,115, as compared with 117,103 for the corresponding period last year.

Telegraphic morbidity reports from State health officers for the week ended Mar. 23, 1946, and comparison with corresponding week of 1945 and 5-year median

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

Division and State	Diphtheria			Influenza			Measles			Meninigitis, meningococcus		
	Week ended—		Median 1941–45	Week ended—		Median 1941–45	Week ended—		Median 1941–45	Week ended—		Median 1941–45
	Mar. 23, 1946	Mar. 24, 1945		Mar. 23, 1946	Mar. 24, 1945		Mar. 23, 1946	Mar. 24, 1945		Mar. 23, 1946	Mar. 24, 1945	
NEW ENGLAND												
Maine	6	0	0	5	1	2	24	1	118	1	2	2
New Hampshire	0	2	0	2	------	1	4	------	18	0	1	0
Vermont	1	0	0	------	------	------	16	7	39	0	0	0
Massachusetts	3	5	1	------	------	------	761	143	782	6	2	8
Rhode Island	2	1	1	1	------	------	4	4	31	1	2	2
Connecticut	1	2	0	3	------	2	185	138	349	3	4	4
MIDDLE ATLANTIC												
New York	14	12	19	[1] 3	[1] 3	[1] 11	4,221	121	2,413	22	32	32
New Jersey	4	6	6	5	6	15	2,591	61	1,515	3	5	5
Pennsylvania	21	12	10	3	3	2	3,949	143	1,206	8	12	12
E. NORTH CENTRAL												
Ohio	18	11	6	4	10	16	571	39	634	6	14	7
Indiana	14	7	7	10	10	23	1,098	37	262	5	5	5
Illinois	54	8	14	30	1	35	1,802	91	1,092	9	10	10
Michigan [2]	11	10	6	1	------	6	3,032	86	904	2	11	11
Wisconsin	5	0	3	70	55	55	1,791	28	1,058	1	4	3
W. NORTH CENTRAL												
Minnesota	7	3	5	------	3	3	45	17	121	3	3	3
Iowa	5	12	3	------	------	------	133	31	239	6	0	0
Missouri	2	6	5	3	11	5	340	6	414	5	6	6
North Dakota	0	1	0	6	12	9	22	6	61	0	0	0
South Dakota	0	0	2	------	------	------	50	23	23	0	1	0
Nebraska	3	3	2	8	15	11	304	26	110	0	0	0
Kansas	4	1	3	2	2	5	1,121	23	760	2	5	5
SOUTH ATLANTIC												
Delaware	0	0	0	------	------	------	44	29	29	1	2	1
Maryland [2]	13	3	3	7	2	6	453	80	196	5	3	5
District of Columbia	0	0	0	------	------	2	214	19	91	5	1	2
Virginia	5	4	4	193	442	480	687	85	692	8	10	10
West Virginia	6	1	3	------	8	20	86	51	280	3	6	2
North Carolina	14	7	8	------	------	63	482	46	1,028	0	9	9
South Carolina	3	6	3	539	260	515	433	22	259	0	0	2
Georgia	1	3	5	261	10	79	306	29	298	0	3	3
Florida	1	4	2	4	------	5	130	35	171	2	7	3
E. SOUTH CENTRAL												
Kentucky	10	5	3	47	------	19	596	9	106	5	6	5
Tennessee	6	6	3	33	50	74	283	135	218	5	8	8
Alabama	6	12	6	124	104	228	141	16	462	8	5	5
Mississippi [2]	13	4	2	------	------	------	------	------	------	1	8	6
W. SOUTH CENTRAL												
Arkansas	5	5	5	109	46	114	172	34	177	3	2	2
Louisiana	16	2	3	88	47	10	233	42	120	10	3	3
Oklahoma	8	5	5	125	68	141	182	44	74	1	0	1
Texas	40	20	37	1,504	1,021	1,049	1,867	650	1,359	8	5	5
MOUNTAIN												
Montana	1	0	1	------	22	17	26	11	53	1	1	0
Idaho	0	1	0	26	------	------	150	1	50	0	0	0
Wyoming	0	0	1	1	------	20	36	16	71	1	0	0
Colorado	8	4	5	29	14	19	637	16	266	0	0	0
New Mexico	2	5	1	4	3	15	9	4	79	0	0	0
Arizona	1	2	2	133	137	138	105	15	53	1	2	0
Utah [2]	0	0	0	1	29	29	655	116	116	1	0	0
Nevada	0	0	0	------	------	------	13	------	10	0	0	0
PACIFIC												
Washington	8	4	2	------	4	6	806	241	241	5	4	4
Oregon	1	36	1	------	16	30	403	52	144	0	1	1
California	25	31	23	93	14	91	3,087	1,226	1,226	9	20	20
Total	368	272	271	3,477	2,429	3,742	34,300	4,055	24,632	166	225	225
12 weeks	4,611	3,713	3,509	173,413	48,724	57,807	186,541	28,015	184,225	2,399	3,016	3,016

[1] New York City only.

[2] Period ended earlier than Saturday.

Telegraphic morbidity reports from State health officers for the week ended Mar. 23, 1946, and comparison with corresponding week of 1945 and 5-year median—Con.

Division and State	Poliomyelitis			Scarlet fever			Smallpox			Typhoid and paratyphoid fever [3]		
	Week ended—		Median 1941–45	Week ended—		Median 1941–45	Week ended—		Median 1941–45	Week ended—		Median 1941–45
	Mar. 23, 1946	Mar. 24, 1945		Mar. 23, 1946	Mar. 24, 1945		Mar. 23, 1946	Mar. 24, 1945		Mar. 23, 1946	Mar. 24, 1945	
NEW ENGLAND												
Maine	0	0	0	33	97	20	0	0	0	0	0	0
New Hampshire	0	0	0	3	3	13	0	0	0	0	0	0
Vermont	1	1	0	2	17	10	0	0	0	0	0	0
Massachusetts	0	2	1	199	380	388	0	0	0	5	2	0
Rhode Island	0	0	0	3	19	16	0	0	0	0	0	0
Connecticut	0	0	0	62	107	81	0	0	0	0	0	0
MIDDLE ATLANTIC												
New York	5	5	1	684	891	587	0	0	0	0	5	5
New Jersey	1	2	0	135	220	220	0	0	0	0	0	1
Pennsylvania	2	2	1	451	797	603	0	0	0	2	6	5
EAST NORTH CENTRAL												
Ohio	2	0	0	409	447	319	0	1	1	3	1	2
Indiana	1	0	0	108	156	156	1	1	1	1	5	3
Illinois	1	1	0	224	482	482	0	1	1	1	1	1
Michigan [2]	0	0	0	148	299	283	0	0	0	0	0	2
Wisconsin	0	0	0	165	307	294	0	0	0	2	1	0
WEST NORTH CENTRAL												
Minnesota	0	0	0	49	137	95	0	0	0	0	0	0
Iowa	0	0	0	67	120	120	2	1	0	0	0	0
Missouri	0	0	0	55	82	125	0	0	0	1	2	2
North Dakota	0	0	0	15	28	23	0	0	0	0	0	0
South Dakota	0	0	0	12	11	24	0	0	0	0	0	0
Nebraska	0	0	0	32	99	54	0	0	0	0	0	0
Kansas	0	1	1	74	87	96	0	0	0	0	0	0
SOUTH ATLANTIC												
Delaware	0	0	0	9	15	15	0	0	0	0	0	0
Maryland [2]	0	0	0	103	291	107	0	0	0	0	0	0
District of Columbia	0	0	0	25	51	23	0	0	0	1	0	0
Virginia	0	1	0	121	149	55	0	0	0	0	0	1
West Virginia	0	0	0	30	54	42	0	0	0	0	2	2
North Carolina	0	2	0	51	111	26	0	0	0	2	1	1
South Carolina	0	0	0	14	6	8	0	0	0	0	0	1
Georgia	0	0	0	6	58	15	1	0	0	5	3	3
Florida	1	0	0	4	7	7	0	0	0	0	1	4
EAST SOUTH CENTRAL												
Kentucky	1	0	0	41	53	63	0	0	0	0	1	1
Tennessee	0	2	0	36	86	64	0	0	1	0	2	1
Alabama	1	1	1	25	14	16	0	0	0	0	1	1
Mississippi [2]	2	1	0	6	22	16	0	0	0	1	1	1
WEST SOUTH CENTRAL												
Arkansas	1	0	0	14	16	15	1	0	0	1	0	2
Louisiana	1	0	0	9	13	10	1	0	0	5	4	4
Oklahoma	0	0	0	18	16	17	0	0	1	1	2	1
Texas	1	4	4	61	94	59	0	0	0	6	3	6
MOUNTAIN												
Montana	1	0	0	10	9	22	0	0	0	0	0	0
Idaho	0	0	0	4	48	6	0	0	0	0	0	0
Wyoming	0	0	0	17	10	10	0	0	0	0	0	0
Colorado	0	0	0	43	71	57	0	0	0	0	0	0
New Mexico	1	0	0	17	23	10	0	0	0	0	5	2
Arizona	0	0	0	17	54	15	0	0	0	1	0	0
Utah [2]	0	0	0	47	30	42	0	0	0	1	0	0
Nevada	0	0	0	1	1	1	0	0	0	0	0	0
PACIFIC												
Washington	1	0	0	27	100	53	7	0	0	2	0	1
Oregon	0	0	0	17	48	19	0	0	0	0	1	1
California	4	3	3	174	388	200	1	0	0	2	0	3
Total	28	28	24	3,877	6,624	4,269	14	4	19	43	50	65
12 weeks	494	425	311	40,402	68,094	48,344	99	118	266	518	674	891

[2] Period ended earlier than Saturday.
[3] Including paratyphoid fever reported separately, as follows: Massachusetts 3; Georgia 1.

Telegraphic morbidity reports from State health officers for the week ended Mar. 23, 1946, and comparison with corresponding week of 1945 and 5-year median—Con.

Division and State	Whooping cough			Week ended Mar. 23, 1946							
	Week ended—		Median 1941–45	Dysentery			Encephalitis, infectious	Rocky Mt. spotted fever	Tularemia	Typhus fever, endemic	Undulant fever
	Mar. 23, 1946	Mar. 24, 1945		Amebic	Bacillary	Unspecified					
NEW ENGLAND											
Maine	30	131	37								1
New Hampshire			3								
Vermont	41	19	19								2
Massachusetts	100	240	232		2						
Rhode Island	36	19	19		1						
Connecticut	65	50	57								
MIDDLE ATLANTIC											
New York	143	232	294	6	4						3
New Jersey	177	101	101	1							
Pennsylvania	138	197	211				1				8
EAST NORTH CENTRAL											
Ohio	48	173	173	1	1						2
Indiana	17	8	34	1					1		4
Illinois	64	55	86	2	1		1		2		1
Michigan [2]	119	100	199	4	3						5
Wisconsin	95	56	101						1		3
WEST NORTH CENTRAL											
Minnesota	5	16	38	1							13
Iowa	18	3	19								
Missouri	7	22	22			1			8		
North Dakota		1	8								
South Dakota	1	1	1								
Nebraska		3	27								
Kansas	19	38	38						1		7
SOUTH ATLANTIC											
Delaware	2	4	4								
Maryland [2]	9	50	50								4
District of Columbia	7	8	8								
Virginia	21	109	74			35					
West Virginia	31	23	23								
North Carolina	59	171	170	1					1	2	
South Carolina	75	46	57	4	8					1	
Georgia	11	19	19		7	25			3	6	3
Florida	11	22	20	2						7	1
EAST SOUTH CENTRAL											
Kentucky	20	18	68					0			
Tennessee	27	168	30						1		1
Alabama	6	14	37						1	5	4
Mississippi [2]									2	1	3
WEST SOUTH CENTRAL											
Arkansas	2	28	20	1					1		
Louisiana	2	2	4	2					2	9	2
Oklahoma	5	12	22								
Texas	194	261	261	8	225	18				11	19
MOUNTAIN											
Montana	2	4	8								1
Idaho	9	4	4								1
Wyoming	2	12	7								
Colorado	39	32	32								
New Mexico	11	2	8			4					
Arizona	17	34	34			17					
Utah [2]	15	27	44								1
Nevada											
PACIFIC											
Washington	28	18	63								2
Oregon	4	24	18	2				1			1
California	90	374	374	1	12		1			1	6
Total	1,822	2,951	3,685	37	264	100	3	1	24	43	98
Same week, 1945	2,951			35	202	118	15	0	8	38	91
Average, 1943–45	2,943			36	188	69	13	[4] 0	12	[4] 37	
12 weeks: 1946	21,802			459	3,459	1,312	97	5	251	576	823
1945	29,090			332	5,912	1,548	88	4	226	613	1,014
Average, 1943–45	32,741		[4] 47,025	324	3,575	937	114	[4] 4	185	[4] 515	

[2] Period ended earlier than Saturday.
[4] 5-year median, 1941–45.

WEEKLY REPORTS FROM CITIES

City reports for week ended Mar. 16, 1946

This table lists the reports from 85 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.

	Diphtheria cases	Encephalitis, infectious, cases	Influenza		Measles cases	Meningitis, meningococcus, cases	Pneumonia deaths	Poliomyelitis cases	Scarlet fever cases	Smallpox cases	Typhoid and paratyphoid fever cases	Whooping cough cases
			Cases	Deaths								
NEW ENGLAND												
Maine:												
Portland	0	0	------	0	2	0	2	0	4	0	0	18
New Hampshire:												
Concord	0	0	------	0	-------	0	0	0	4	0	0	0
Vermont:												
Barre	0	0	------	0	-------	0	0	0	0	0	0	------
Massachusetts:												
Boston	5	0	------	0	183	1	18	0	38	0	0	23
Fall River	0	0	------	0	3	0	2	0	5	0	0	1
Springfield	0	0	------	0	14	0	0	0	6	0	0	3
Worcester	0	0	------	0	42	0	12	0	3	0	0	27
Rhode Island:												
Providence	2	0	1	0	5	0	0	0	5	0	0	30
Connecticut:												
Bridgeport	0	0	1	1	-------	1	0	0	1	0	0	1
New Haven	0	0	------	0	90	0	2	0	2	0	0	3
MIDDLE ATLANTIC												
New York:												
Buffalo	1	0	------	1	221	1	6	0	20	0	1	11
New York	15	0	4	2	976	11	67	0	378	0	0	29
Rochester	0	1	------	0	409	0	2	0	14	0	0	1
Syracuse	0	0	------	0	293	0	1	1	12	0	0	1
New Jersey:												
Camden	0	0	------	0	70	0	1	0	0	0	0	8
Newark	0	0	1	0	760	0	3	0	16	0	0	14
Trenton	0	0	1	0	7	1	0	0	1	0	0	3
Pennsylvania:												
Philadelphia	0	0	5	4	985	3	25	0	60	0	1	15
Pittsburgh	0	0	2	3	3	2	5	0	8	0	0	------
Reading	0	0	------	0	538	0	4	0	3	0	0	13
EAST NORTH CENTRAL												
Ohio:												
Cincinnati	1	0	1	1	118	1	9	0	20	0	0	2
Cleveland	2	0	3	1	29	2	8	0	31	0	0	6
Columbus	0	0	------	0	3	0	0	0	12	0	0	------
Indiana:												
Fort Wayne	1	0	------	0	5	0	2	0	1	0	0	------
Indianapolis	0	0	------	0	628	0	7	0	11	0	0	3
South Bend	0	0	------	0	-------	0	0	0	2	0	0	------
Terre Haute	0	0	------	0	1	0	0	0	4	0	0	1
Illinois:												
Chicago	1	0	4	0	987	5	34	0	76	0	0	50
Michigan:												
Detroit	10	1	------	1	1,470	1	7	0	38	0	0	------
Flint	1	0	------	0	7	0	5	0	2	0	0	------
Grand Rapids	0	0	------	0	97	0	0	0	4	0	0	1
Wisconsin:												
Kenosha	0	0	------	0	2	0	0	0	5	0	0	------
Milwaukee	0	0	1	1	669	2	5	0	30	0	0	20
Racine	0	0	------	0	12	0	0	0	1	0	0	1
Superior	0	0	------	0	2	0	0	0	1	0	0	------
WEST NORTH CENTRAL												
Minnesota:												
Duluth	0	0	------	1	13	0	2	0	3	0	0	3
Minneapolis	4	0	------	0	17	0	4	0	5	0	0	2
Missouri:												
Kansas City	0	0	------	0	167	1	11	0	2	0	0	------
St. Joseph	1	0	------	0	12	0	0	0	1	0	1	------
St. Louis	5	0	1	0	57	1	11	1	24	0	0	

City reports for week ended Mar. 16, 1946—Continued

	Diphtheria cases	Encephalitis, infectious, cases	Influenza		Measles cases	Meningitis, meningococcus, cases	Pneumonia deaths	Poliomyelitis cases	Scarlet fever cases	Smallpox cases	Typhoid and paratyphoid fever cases	Whooping cough cases
			Cases	Deaths								
WEST NORTH CENTRAL—continued												
Nebraska:												
Omaha	2	0	------	0	57	0	4	0	13	0	0	2
Kansas:												
Wichita	0	0	1	0	47	0	4	0	6	0	0	4
SOUTH ATLANTIC												
Delaware:												
Wilmington	0	0	------	0	16	0	1	0	1	0	0	------
Maryland:												
Baltimore	10	1	1	0	305	2	12	0	40	0	1	15
Cumberland	0	0	------	0	-------	0	0	0	4	0	0	------
Frederick	0	0	------	0	-------	0	0	0	0	0	1	------
District of Columbia:												
Washington	0	0	------	0	179	5	10	0	30	0	1	2
Virginia:												
Lynchburg	0	0	------	1	6	0	1	0	0	0	0	2
Richmond	0	0	------	0	23	0	1	0	9	0	0	3
Roanoke	0	0	------	0	20	0	0	0	5	0	0	------
West Virginia:												
Charleston	0	0	------	0	1	0	0	0	2	0	0	------
Wheeling	0	0	------	0	5	0	1	0	0	0	0	7
North Carolina:												
Raleigh	0	0	------	0	48	0	0	0	0	0	0	1
Wilmington	0	0	------	0	17	0	0	0	0	0	0	1
Winston-Salem	0	0	------	0	16	0	2	0	4	0	0	15
South Carolina:												
Charleston	0	0	17	0	14	0	3	0	0	0	0	4
Georgia:												
Atlanta	0	0	11	0	2	0	6	0	3	0	0	------
Brunswick	0	0	------	0	1	0	0	0	0	0	0	------
Savannah	0	0	3	1	1	0	2	0	0	0	0	------
Florida:												
Tampa	0	0	------	0	31	0	7	0	1	0	0	------
EAST SOUTH CENTRAL												
Tennessee:												
Memphis	0	0	1	1	30	1	8	0	5	0	0	3
Nashville	0	0	------	0	16	0	1	0	0	0	0	------
Alabama:												
Birmingham	0	0	6	0	9	0	1	0	1	0	0	2
Mobile	0	0	------	2	1	0	1	0	0	0	0	------
WEST SOUTH CENTRAL												
Arkansas:												
Little Rock	0	0	------	0	6	0	2	0	1	0	0	------
Louisiana:												
New Orleans	*18	0	11	1	5	5	*5	1	14	0	1	------
Shreveport	0	0	------	0	-------	0	4	0	1	0	0	------
Texas:												
Dallas	2	0	------	0	5	0	6	0	5	0	0	------
Galveston	0	0	------	0	5	0	3	0	0	0	1	------
Houston	5	0	------	0	-------	0	3	2	0	0	0	------
San Antonio	1	0	4	5	27	1	3	0	1	0	0	1
MOUNTAIN												
Montana:												
Billings	0	0	------	0	-------	0	1	1	0	0	0	------
Great Falls	1	0	------	0	1	0	3	0	1	0	0	------
Helena	0	0	------	0	-------	0	0	0	0	0	0	------
Missoula	0	0	------	0	-------	0	0	0	0	0	0	------
Idaho:												
Boise	0	0	------	0	5	0	0	0	0	0	0	------
Colorado:												
Denver	2	0	4	0	256	1	10	0	10	0	0	14
Pueblo	1	0	------	0	5	0	1	0	2	0	0	1
Utah:												
Salt Lake City	0	0	------	1	91	0	2	0	2	0	0	6

*Including monthly report from Charity Hospital, New Orleans (data excluded in computing rates).

City reports for week ended Mar. 16, 1946—Continued

	Diphtheria cases	Encephalitis, infectious, cases	Influenza		Measles cases	Meningitis, meningococcus, cases	Pneumonia deaths	Poliomyelitis cases	Scarlet fever cases	Smallpox cases	Typhoid and paratyphoid fever cases	Whooping cough cases
			Cases	Deaths								
PACIFIC												
Washington:												
Seattle	2	0	------	1	211	1	4	0	11	4	1	7
Spokane	0	1	------	0	138	0	2	0	0	0	0	1
Tacoma	0	0	------	0	24	0	0	0	1	0	0	7
California:												
Los Angeles	2	0	17	0	289	3	3	0	58	0	0	13
Sacramento	0	0	1	1	140	1	3	0	1	0	0	1
San Francisco	1	0	3	0	282	2	8	1	13	0	1	4
Total	78	4	105	30	11,232	55	378	7	1,098	4	10	407
Corresponding week, 1945	69	------	46	27	861	------	454	------	1,906	0	11	655
Average, 1941–45	65	------	216	[1] 39	[2] 6,250	------	[1] 481	------	1,758	1	13	799

[1] 3-year average, 1943–45.
[2] 5-year median, 1941–45.

Anthrax.—Cases: Philadelphia, 1.
Dysentery, amebic.—Cases: New York, 5; Detroit, 1; Los Angeles, 1.
Dysentery, bacillary.—Cases: Baltimore, 1; Charleston, S. C., 3; Los Angeles, 1; San Francisco, 1.
Dysentery, unspecified.—Cases: San Antonio, 3.
Leprosy.—Cases: Tampa, 1.
Tularemia.—Cases: Winston-Salem, 1; New Orleans, 1.
Typhus fever, endemic.—Cases: New York, 1; New Orleans, 19; Los Angeles, 1.

Rates (annual basis) per 100,000 population, by geographic groups, for the 85 cities in the preceding table (estimated population, 1943, 33,776,800)

	Diphtheria case rates	Encephalitis, infectious, case rates	Influenza		Measles case rates	Meningitis, meningococcus, case rates	Pneumonia death rates	Poliomyelitis case rates	Scarlet fever case rates	Smallpox case rates	Typhoid and paratyphoid fever case rates	Whooping cough case rates
			Case rates	Death rates								
New England	20.0	0.0	5.7	2.9	971	5.7	103.1	0.0	195	0.0	0.0	304
Middle Atlantic	7.4	0.5	6.0	4.6	1,973	8.3	52.8	0.5	237	0.0	0.9	44
East North Central	9.8	0.6	5.5	2.5	2,471	6.7	47.2	0.0	146	0.0	0.0	52
West North Central	27.8	0.0	4.6	2.3	857	4.6	83.4	2.3	125	0.0	2.3	28
South Atlantic	16.3	1.6	52.3	3.3	1,120	11.4	75.2	0.0	162	0.0	4.9	82
East South Central	0.0	0.0	41.3	17.7	331	5.9	64.9	0.0	35	0.0	0.0	30
West South Central	32.4	0.0	43.0	17.2	138	17.2	85.0	8.6	63	0.0	5.7	3
Mountain	31.8	0.0	31.8	7.9	2,843	7.9	135.0	7.9	119	0.0	0.0	167
Pacific	7.9	1.6	33.2	3.2	1,714	11.1	31.6	1.6	133	6.3	3.2	52
Total	12.3	0.6	16.3	4.6	1,739	8.5	59.4	1.1	170	0.6	1.5	63

SMALLPOX IN SAN FRANCISCO, CALIF., AND SEATTLE, WASH.

Smallpox has recently appeared on the West Coast in San Francisco and Seattle, apparently introduced from the Orient in both instances. The first case in San Francisco occurred in a patient who arrived from Japan on December 28, the eruption appearing on December 29. Up to March 27 a total of 7 cases had been reported in San Francisco.

The first case in Seattle was reported with onset on February 19, following exposure, on February 5, to a case in a soldier who had returned from the Orient. Up to March 26, 15 cases and 2 suspect cases, traceable to the original cases, had been reported in Seattle and King County.

Intensive immunization is reported under way in both cities.

TERRITORIES AND POSSESSIONS

Hawaii Territory

Plague (rodent).—A rat found on January 17, 1946, in District 15A, Hamakua Mill area, Honokaa, Hamakua District, Island of Hawaii, T. H., was proved positive for plague on January 28, 1946.

FOREIGN REPORTS

CANADA

Provinces—Communicable diseases—Week ended February 23, 1946.—During the week ended February 23, 1946, cases of certain communicable diseases were reported by the Dominion Bureau of Statistics of Canada as follows:

Disease	Prince Edward Island	Nova Scotia	New Brunswick	Quebec	Ontario	Manitoba	Saskatchewan	Alberta	British Columbia	Total
Chickenpox		8	4	105	322	47	23	36	103	648
Diphtheria		4	2	25	5	7				4
Dysentery, bacillary				4	1				7	1
Encephalitis, infectious					1					1
German measles		3		20	28			7	10	68
Influenza		132			104	3			158	397
Measles		157	22	482	1,716	1	4	27	47	2,456
Meningitis, meningococcus					3				1	4
Mumps			4	36	322	47	10	38	125	582
Scarlet fever		14	8	33	96	13	2	15	15	196
Tuberculosis (all forms)		1	2	126	48	7	3	25	89	301
Typhoid and paratyphoid fever		5		7	6			3		21
Undulant fever			1		1					2
Venereal diseases:										
Gonorrhea		20	7	57	156	33	41	46	71	431
Syphilis		7	6	145	129	7	10	12	36	352
Other forms				1						1
Whooping cough		11		58	68	2		6		145

FINLAND

Notifiable diseases—January 1946.—During the month of January 1946, cases of certain notifiable diseases were reported in Finland as follows:

Disease	Cases	Disease	Cases
Actinomycosis	1	Mumps	872
Cerebrospinal meningitis	15	Ophthalmia neonatorum	2
Chickenpox	1,541	Paratyphoid fever	203
Conjunctivitis	19	Pneumonia (all forms)	2,758
Diphtheria	1,553	Poliomyelitis	25
Dysentery, unspecified	11	Puerperal fever	41
Gastroenteritis	2,437	Rheumatic fever	334
Gonorrhea	1,736	Scabies	6,156
Hepatitis, epidemic	1,000	Scarlet fever	299
Influenza	1,316	Syphilis	671
Laryngitis	32	Typhoid fever	94
Malaria	1	Vincent's angina	20
Measles	61	Whooping cough	1,015

POLAND

Principal communicable diseases.—UNRRA reports new cases of certain communicable diseases in Poland during January 1946 as follows: Diphtheria, 2,190; scarlet fever, 771; typhoid fever, 6,624; typhus fever, 1,066.

The Ministry of Health estimates that there are 1,200,000 cases of active tuberculosis in Poland; that 70 percent of hospital effectiveness has been destroyed by war; and that there are only about 6,000 physicians in the entire country as compared with 13,000 before the war.

REPORTS OF CHOLERA, PLAGUE, SMALLPOX, TYPHUS FEVER, AND YELLOW FEVER RECEIVED DURING THE CURRENT WEEK

NOTE.—Except in cases of unusual incidence, only those places are included which had not previously reported any of the above-mentioned diseases, except yellow fever, during recent months. All reports of yellow fever are published currently.

A table showing the accumulated figures for these diseases for the year to date is published in the PUBLIC HEALTH REPORTS for the last Friday in each month.

Smallpox

Morocco (French).—For the period March 1–10, 1946, 127 cases of smallpox were reported in French Morocco. Regions reporting the highest incidence are: Agadir and frontier districts, 13; Casablanca, 35; Fez, 2; Marrakech, 55; Meknes, 9; Oujda, 4; Rabat, 9.

Typhus Fever

Belgian Congo.—For the week ended March 2, 1946, 96 cases of typhus fever were reported in Belgian Congo.

Egypt.—For the week ended February 16, 1946, 81 cases of typhus fever with 4 deaths were reported in all of Egypt.

Eritrea.—For the week ended March 2, 1946, 26 cases of typhus fever were reported in Eritrea.

Guatemala.—For the month of January 1946, 76 cases of typhus fever with 13 deaths were reported in Guatemala. Departments reporting the highest incidence are: Huehuetenango, 20 cases, 5 deaths; Totonicapan, 16 cases, 1 death; Chimaltenango, 15 cases, 3 deaths; Quezaltenango, 15 cases, 3 deaths.

Morocco (French).—For the period March 1–10, 1946, 195 cases of typhus fever were reported in French Morocco. Regions reporting the highest incidence are: Casablanca, 72; Fez, 24; Marrakech, 23; Meknes, 42; Oujda, 2; Rabat, 32.

Turkey.—For the week ended March 16, 1946, 88 cases of typhus fever were reported in Turkey, including cases reported in ports as follows: Icel, 1; Istanbul, 6; Zonguldak, 1.