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THE OFFICIAL UNITED STATES AND INTERNATIONAL UNIT FOR STANDARDIZING GAS GANGRENE ANTITOXIN (HISTOLYTICUS)

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The work of standardizing gas gangrene antitoxin (*histolyticus*) has been conducted in a manner similar to that employed in the standardization of the other gas gangrene antitoxins (*perfringens*, *Vibrion septique* and *odematiens*). The undertaking has been a cooperative effort on the part of various laboratories. The initial planning of the experiments, and the preparation of the necessary materials for the international testing have been carried out by Drs. Walbum and Reymann in the laboratory of Dr. Th. Madsen, of the State Serum Institute of Copenhagen, Denmark, in accordance with the recommendation of the Permanent Commission on Biological Standardization at the meeting held in Copenhagen in November 1932.

The laboratories participating in the tests were the following:

Istituto Bacteriologico, Argentina, South America.
Pasteur Institute, Paris, France.
Institut für Experimentelle Therapie "Emil von Behring", Marburg-am-Lahn, Germany.
National Institute for Medical Research, Hampstead, London, England.
Wellcome Physiological Research Laboratories, Beckenham, Kent, England.
Lister Institute of Preventive Medicine, Elstree, Herts, England.
State Institute "L. A. Tarassevitch", Moscow, U. S. S. R.
National Institute of Health, Washington, D. C.

The standard preparations for carrying out the tests were received from Dr. Madsen in June 1935. These consisted of 1 ampul of *histolyticus* toxin (A/34), 1 bottle of glycerinated *histolyticus* antitoxin (the provisional standard), and one bottle of *histolyticus* antitoxin H of unstated potency.

At the time of the receipt of the reagents the National Institute of Health had on hand a dried *histolyticus* serum which it was intended to use as the American standard *histolyticus* antitoxin. As shown later in this paper, this antitoxin was standardized in terms of the provi-

sional international unit proposed by Walbum and Reymann through the *histolyticus* test toxin A/34 furnished by Dr. Madsen. The National Institute of Health test toxin was prepared later. (See the following paper by S. E. Stewart.)

The international provisional unit proposed by Walbum and Reymann was of the same dimensions as the unit introduced by Weinberg of the Pasteur Institute. This unit was used as the basis of their standardization studies and was designated as the P unit in the tests.

The glycerinated antitoxin received had been diluted so that 1 cc of the solution contained 20 provisional units. (The average weights of 8 ampuls containing the dried residue of 5 cc of serum in each ampul was 0.4966 gram. This amount represented 1,389 P units, and 1 P unit was therefore contained in 0.3575 milligram of the dried serum. By diluting the contents of 2 ampuls to 138.90 cc of a mixture of physiological salt solution (34 percent) and glycerin (66 percent), 20 P units were contained in 1 cc.)

It was recommended that the correctness of the assay of the toxin and antitoxin made by the authors be checked by (*a*) determination of the "test-dose" of the toxin against the standard antitoxin by means of intravenous injection into mice, and (*b*) assay of the antitoxin against this "test-dose" of toxin by intravenous injection of mice with mixtures of the "test-dose" of toxin with the amount of antitoxin used in determining the "test-dose" of toxin as well as amounts of antitoxin 10 percent above and 10 percent below this figure.

It was suggested that the standard antitoxin be diluted so that 1 cc of the solution would contain 5 P units and that the toxin be diluted so that 1 cc would contain 10 mg of toxin. The mixtures of standard antitoxin and toxin solution were prepared in such a manner that the dose of the mixture injected did not exceed 0.5 cc. A 3-day period of observation of the animals was recommended.

The tests suggested were carried out using the reagents submitted, and similar tests were carried out with our own standard antitoxin.

I. TESTS WITH INTERNATIONAL REAGENTS

(*a*) *Determination of the "test-dose" of toxin A/34.*—The toxin A/34 was tested against one unit of the international provisional standard, with the results shown in table 1. The mixture of the toxin and antitoxin was contained in 0.5 cc (0.2 cc of the antitoxin dilution (=1 P unit) and 0.195 to 0.255 cc of the toxin dilution plus sufficient normal salt solution to equal 0.5 cc). The results show a "test-dose" of 2.4 mg of the toxin, the value being slightly higher than that found by Walbum and Reymann, which might be accounted for by a slight deterioration of the toxin.

TABLE 1.—*Determination of the test dose of toxin A/34*

P. units antitoxin	Toxin A/34 milligrams	Number of mice	Mice surviving	
			Number	Proportion
1.0	1.95	6	6	6/6
1.0	2.10	6	6	6/6
1.0	2.25	6	6	6/6
1.0	2.40	6	3	3/6
1.0	2.55	6	2	2/6

(*b*) *Assay of the international provisional standard antitoxin.*—In order to check the titration of the toxin, the "test-dose" of toxin was tested against 1 unit of the international standard antitoxin and also against amounts of the antitoxin 10 percent above and 10 percent below 1 unit. The results as shown in table 2 confirm the results obtained in the determination of the "test-dose" of toxin.

TABLE 2.—*Assay of the provisional international histolyticus antitoxin*

P. units antitoxin	Toxin A/34 milligrams	Number of mice	Mice surviving	
			Number	Proportion
1.1	2.4	6	6	6/6
1.0	2.4	6	3	3/6
0.9	2.4	6	0	0/6

(*c*) *Titration of histolyticus antitoxin H of unstated potency.*—In the memorandum accompanying the reagents received from Dr. Madsen it was stated that the potency of the *histolyticus* antitoxin H lay between 200 and 400 units. For the preliminary tests a potency around 300 units per cubic centimeter was assumed. A 1/60 dilution of the antitoxin was made, so that 1 cc contained 5 of the assumed units and 0.2 cc of this dilution was equivalent to 1 unit. Titrations were made against the "test-dose" of toxin (2.4 mg). The results are shown in the accompanying protocol, table 3.

TABLE 3.—*Assay of international histolyticus antitoxin H*

P units tested for	Equivalent units per cc	Toxin A/34 milligrams	Number of mice	Mice surviving	
				Number	Proportion
1.5	200	2.4	6	6	6/6
1.3	230	2.4	6	6	6/6
1.2	250	2.4	6	5	5/6
1.1	272	2.4	6	2	2/6
1.0	300	2.4	6	0	0/6
0.9	333	2.4	6	0	0/6
0.8	374	2.4	6	0	0/6
0.75	400	2.4	6	0	0/6

A unitage in the neighborhood of 272 is indicated by the results of the test. In a second test the doses of antitoxin were spaced at closer intervals. The results are shown in table 4.

TABLE 4.—*Assay of histolyticus antitoxin H*

P units tested for	Equivalent units per cc	Toxin A/34 milligrams	Number of mice	Mice surviving	
				Number	Proportion
1	300	2.4	6	1	1/6
1.035	290	2.4	6	1	1/6
1.072	280	2.4	6	2	2/6
1.1	270	2.4	6	3	3/6
1.154	260	2.4	6	5	5/6
1.2	250	2.4	6	6	6/6

The results indicate a unitage of approximately 270–280 per cubic centimeter.

The reports of the various laboratories collaborating in the tests were presented at the meeting of the Permanent Commission on Biological Standardization in Geneva on September 30, 1935. The results of the testing of the antitoxin of unstated potency by the various participants in the project were in close agreement, as shown in the following tabulation:

	Units
Argentina: Istituto Bacteriologico	275–300
France: Pasteur Institute	300–350
Germany: Institut für Experimentelle Therapie "Emil von Behring"	250
Great Britain: National Institute for Medical Research	285
Wellcome Physiological Research Laboratories	270–300
Lister Institute of Preventive Medicine	285
United States of America: National Institute of Health	270–280
U. S. S. R.: State Institute "L. A. Tarassevitch"	275

II. TESTS WITH AMERICAN REAGENTS

STANDARD ANTITOXIN

The *histolyticus* serum used as the American standard was obtained from the Lederle Laboratories, Inc. It was received without preservative and was measured accurately soon after receipt in 10 cc amounts into 30-cc pyrex glass ampuls. After thorough drying over phosphorus pentoxide, a small agglutination tube containing phosphorus pentoxide was placed in each ampul. The air was evacuated and replaced by nitrogen, and the ampul was sealed.

The weights of the dried residue contained in 8 ampuls were determined with the following results: 0.9451 gram, 0.9442 gram, 0.9424 gram, 0.9456 gram, 0.9476 gram, 0.9431 gram, 0.9446 gram, 0.9445 gram. The average weight was 0.9445 gram, and the largest deviation from the mean was 0.32 percent.

The dried serum of one of the ampuls was dissolved and titrated against the "test-dose" of the toxin A/34 received from Dr. Madsen.

The dried serum was dissolved in 50 cc of saline, and from this dilutions were made up to 1/2000 for the preliminary test. The results are shown in table 5.

TABLE 5.—*Assay of the American standard histolyticus antitoxin against 2.4 mg of toxin A/34*

Preliminary test

Dilution of antitoxin	Amount of dilution	Number of mice used	Mice surviving	
			Number	Proportion
	cc			
1/50	0.2	3	3	3/3
1/100	.2	3	3	3/3
1/200	.2	3	3	3/3
1/500	.2	3	3	3/3
1/1000	.2	3	0	0/3
1/1500	.2	3	0	0/3
1/2000	.2	3	0	0/3

Dilutions were then made between 1/500 and 1/1000. The results are given in table 6.

TABLE 6.—*Assay of the American standard histolyticus antitoxin against 2.4 mg of toxin A/34*

Second test

Dilution of antitoxin	Amount of dilution	Number of mice used	Mice surviving	
			Number	Proportion
	cc			
1/500	0.2	3	3	3/3
1/600	.2	3	3	3/3
1/700	.2	3	2	2/3
1/800	.2	3	1	1/3
1/900	.2	3	0	0/3
1/1000	.2	3	0	0/3

From the results obtained it was assumed that 0.2 cc of the 1/850 dilution of the American Standard antitoxin was equivalent to 1 unit. Varying amounts of the 1/850 dilution were then tested against the "test-dose" of toxin A/34 with the following results:

TABLE 7.—*Assay of the American standard histolyticus antitoxin against 2.4 mg of toxin A/34*

Third test

Amount of 1/850 dilution of antitoxin	Number of mice used	Mice surviving		Amount of 1/850 dilution of antitoxin	Number of mice used	Mice surviving	
		Number	Proportion			Number	Proportion
0.24 cc	6	5	5/6	0.20 cc	6	0	0/6
0.23 cc	6	4	4/6	0.19 cc	6	0	0/6
0.22 cc	6	1	1/6	0.18 cc	6	0	0/6
0.21 cc	6	0	0/6				

From table 7 it can be seen that 0.23 cc of 1/850 dilution of the American standard antitoxin (equivalent to 0.2 cc of a 1/739 dilution) gave the most satisfactory results; and 0.2 cc of a 1/739 dilution of antitoxin was therefore considered as containing one unit.

The results show that 1 cc of a 1/739 dilution of the American *histolyticus* serum is equivalent to 1 cc of a 1/555.6 dilution of the international serum (1 cc of 1/138.9 diluted 1 to 4). Since it was considered desirable to dilute the glycerinated antitoxin in such a way that it would be diluted 1 to 10 in the final testing instead of 1 to 4 as the glycerinated international standard was diluted, the contents of one ampul were dissolved in 73.9 cc of a mixture of 66 percent glycerin and 34 percent normal salt solution so that 1 cc contained 50 units. One cubic centimeter of a 1/10 dilution of this glycerinated serum contains 5 units. The comparison between the international standard and the American standard may be expressed thus:

International standard antitoxin: 1 cc of 1/138.9×1/4 dilution contains 5 units.

American standard antitoxin: 1 cc of 1/73.9×1/10 dilution contains 5 units.

On the basis of the mean weight of the dried residue of 10 cc of the standard antitoxin (0.9445 gram) this amount contains 3,695 units and 1 unit is contained in 0.2556 mg of the standard antitoxin. This amount is therefore equivalent to 0.3575 mg of the dried international standard.

The American antitoxin diluted as indicated by the above results was tested against the international toxin A/34. One unit of antitoxin and amounts 10 percent above and 10 percent below one unit were tested against the "test dose", 2.4 mg of toxin. The results show that the antitoxin was correctly diluted, since one unit of antitoxin allowed four out of six mice to survive (table 8).

TABLE 8.—*Assay of American histolyticus antitoxin*

P units	Toxin A/34	Number of mice used	Mice surviving	
			Number	Proportion
	Mg			
1.1	2.4	6	6	6/6
1.0	2.4	6	4	4/6
0.9	2.4	6	0	0/6

STANDARD TOXIN

A dried *histolyticus* toxin was prepared as described in the following paper. This toxin was titrated against the American standard antitoxin and the "test-dose" determined. Titrations were made by the

methods of intravenous injection of mice and intracutaneous injections of guinea pigs.

(*a*) *Determination of the "test-dose" of the standard toxin on mice.*—The dried toxin which had an M. L. D. of 0.02 mg was tested against the American standard antitoxin using 40, 45, and 50 M. L. D. against 1 unit of the antitoxin. The toxin was diluted so that 1 cc contained 10 mg of toxin. The results are given in table 9.

TABLE 9.—*Determination of the "test-dose" of American histolyticus toxin A*

[Antitoxin constant (1 unit); toxin varied]

Units	Toxin	Number of mice used	Mice surviving	
			Number	Proportion
	Mg			
1.0	0.8	6	6	6/6
1.0	.9	6	3	3/6
1.0	1.0	6	0	0/6

The "test-dose" of the toxin was found to be 0.9 mg. For a further check on the "test-dose" the toxin was titrated against varying amounts of antitoxin with the toxin constant (0.9 mg). (Table 10.)

TABLE 10.—*Determination of the "test-dose" of the toxin*

[Antitoxin varied; toxin constant (0.9 mg)]

Units	Toxin	Number of mice used	Mice surviving	
			Number	Proportion
	Mg			
1.1	0.9	6	5	5/6
1.0	.9	6	2	2/6
.9	.9	6	0	0/6

To check further the "test-dose" of the American standard toxin, it was tested against the international *histolyticus* antitoxin H of unstated potency. As has been previously shown, this antitoxin was found to contain between 270 to 280 units per cc when tested against the "test-dose" (2.4 mg) of the international toxin. Taking 275 units per cc as the strength of the toxin, a 1/55 dilution was made so that 0.2 cc contained 1 unit, and this was tested against the "test-dose" (0.9 mg) of the American toxin. Table 11 gives the results.

TABLE 11.—*Assay of histolyticus antitoxin H*

Units antitoxin H	American standard toxin	Number of mice used	Mice surviving	
			Number	Proportion
	Mg.			
0.9	0.9	6	0	0/6
1.0	.9	6	2	2/6
1.1	.9	6	6	6/6

(b) *Intracutaneous tests on guinea pigs.*—The intracutaneous test on guinea pigs for determining the "test-dose" of toxin was found to give very satisfactory and clear-cut results. The same dilutions used in the mouse intravaneous test were found applicable to the guinea pig intracutaneous test. The mixtures, however, were used in 0.2 cc amounts instead of 0.5 cc as in the mouse test, the 0.2 cc of the mixture containing 0.4 of a unit of antitoxin. White or yellow guinea pigs weighing from 300 to 400 grams were used. Readings were made at the end of 48 hours. The results obtained in titrating the toxin against a constant amount of antitoxin are shown in table 12.

TABLE 12.—*Intracutaneous testing on guinea pigs. Determination of "test-dose" of toxin*

[Antitoxin constant; toxin varied]

Toxin	Antitoxin	Reaction after 48 hours
Mg.	*Unit*	
0.32	0.4	+
.36	.4	++
.4	.4	+++

+++ large reaction; necrosis.
++ moderate reaction; slight necrosis.
+ small reaction.

The results obtained were checked by testing varying doses of antitoxin against the test dose of the toxin. The results are given in table 13.

TABLE 13.—*Intracutaneous testing on guinea pigs. Determination of the "test-dose" of toxin*

[Antitoxin varied; toxin constant (0.36 mg)]

Toxin	Antitoxin	Reaction after 48 hours
Mg	*Unit*	
0.36	0.36	+++
.36	.4	++
.36	.44	+

The slight reaction given by the smallest dose of toxin consisted of a small inflamed reddened area about 0.25 cm in diameter. The next dose, the one giving the ++ reaction which was adopted as the "test-dose" of the toxin showed a larger inflamed area about 1 cm in size with slight necrosis. The reaction produced by the largest dose showed extreme inflammation and marked necrosis.

The results attained by the intracutaneous test agree very well with those obtained by the mouse intravaneous test.

(c) *Potency of commercial and other antitoxins.*—Several antitoxins were available for testing. These included three commercial anti-

toxins all monovalent, one from Dr. Sordelli of the Argentine Republic and one from the Pasteur Institute. These were tested against the "test-dose" of the United States toxin with the following results:

1. Below 20 units per cubic centimeter.
2. 100 units per cubic centimeter.
3. Below 12 units per cubic centimeter.
4. 800 units per cubic centimeter.
5. 100 units per cubic centimeter.

In accordance with the international agreement regarding the size of the unit, the following statement was issued to the various biologics firms in this country:

NATIONAL INSTITUTE OF HEALTH,
25TH AND E STREETS NW.,
WASHINGTON, D. C., *July 6, 1936.*

It is proposed to adopt as the official unit for the measurement of the potency of *histolyticus* antitoxin the equivalent of the International Unit adopted by the Permanent Commission on Biological Standardization of the Health Organization of the League of Nations, this unit being that amount of antitoxin contained in a specified amount of the International serum. The equivalent of the International Unit is that amount of antitoxin contained in 0.2556 milligram of the dried standard serum prepared at the National Institute of Health. The dried serum as dissolved and diluted for distribution contains 50 units in 1 cc.

The standard unit will be distributed on special request addressed to the Director of the National Institute of Health.

It is expected that this unit will be employed by all producers not later than November 1, 1936.

G. W. McCOY,
Director, National Institute of Health.

SUMMARY

The international unit for measuring the potency of gas gangrene antitoxin (*histolyticus*) adopted at a meeting of the Permanent Standards Commission of the Health Organization of the League of Nations in September 1935, at Geneva, has been adopted as the American unit.

The National Institute of Health collaborated with other foreign institutions in checking the assay of the international standard reagents, prepared in the laboratory of the State Serum Institute at Copenhagen. Tests to determine the strength of a specimen of antitoxin of unknown potency by the eight laboratories participating in the project show close agreement.

A standard antitoxin for use in this country has been prepared and its potency measured in terms of the international standard. One unit of the international standard antitoxin contained in 0.3575 mg of the dried serum is equivalent to 0.2556 mg of the United States dried serum. Glycerinated solutions of our standard are prepared in such a manner that 1 cc contains 50 units.

A dried toxin was prepared and the "test-dose" determined against 1 unit of the United States standard antitoxin. The "test-dose" was 0.9 mg of toxin (approximately 45 minimal lethal doses).

Tests are carried out by the intravenous inoculation of mice or the intracutaneous inoculation of guinea pigs. In control tests with the standard antitoxin, 1 unit of antitoxin is tested against the test dose of toxin in mice. The same mixtures may be used in the intracutaneous tests on guinea pigs, employing a dose of 0.4 unit of antitoxin against 0.4 of the "test-dose" of toxin.

STUDIES ON THE PRODUCTION OF TOXIN BY CLOSTRIDIUM HISTOLYTICUM

By SARAH E. STEWART, *Assistant Bacteriologist, National Institute of Health, United States Public Health Service*

This paper is concerned with experimental work in the production of a potent *histolyticus* toxin with particular reference to the influence of the reaction of the medium, the length of the incubation period, the effect of the addition of the glucose, and the results obtained by the use of two different peptones, Parke-Davis and Witte.

Twenty-three strains of *Clostridium histolyticum* were tested for their virulence in mice by intravenous inoculations, and of these the most virulent was selected and used for toxin production. This strain was H 32, received from Dr. R. S. Spray, of the University of West Virginia Medical School.

INFLUENCE OF THE HYDROGEN ION CONCENTRATION OF THE MEDIUM

A relatively strong toxin was obtained by culturing the bacillus in 1-percent Parke-Davis meat infusion broth with a pH of 7.6. At the beginning of the work the pH of the medium seemed to be of considerable importance. With media having pH values above 7.4 the toxin produced would be increasingly weaker the more alkaline the media. Later, however, it was found that a variation in pH from 6.8 to 7.8 gave little difference in the strength of the toxin produced when the medium was suitable in other respects and when conditions of anaerobiosis were favorable.

PERIOD OF INCUBATION

The period of incubation was found to be of considerable importance, 13 to 15 hours giving the maximum toxin production. With an increase in the period of incubation, a decrease in toxicity was observed; this increase in the incubation was accompanied by an increase in alkalinity. This is illustrated in figure 1. The optimum period of incubation, however, seems to vary with the type of medium

used. Mita (*1*), with a liver infusion broth, obtained the most potent toxin after 24 hours' incubation.

EFFECT OF ADDITION OF GLUCOSE TO THE MEDIUM

Although *Cl. histolyticum* is nonsaccharolytic, Weinberg and Randin (*2*) were able to show that if 2 percent glucose were added to the medium a stronger toxin would be produced. Their work has been confirmed in these studies. To demonstrate the effect of glucose on toxin production, a sugar-free meat infusion broth (coli-

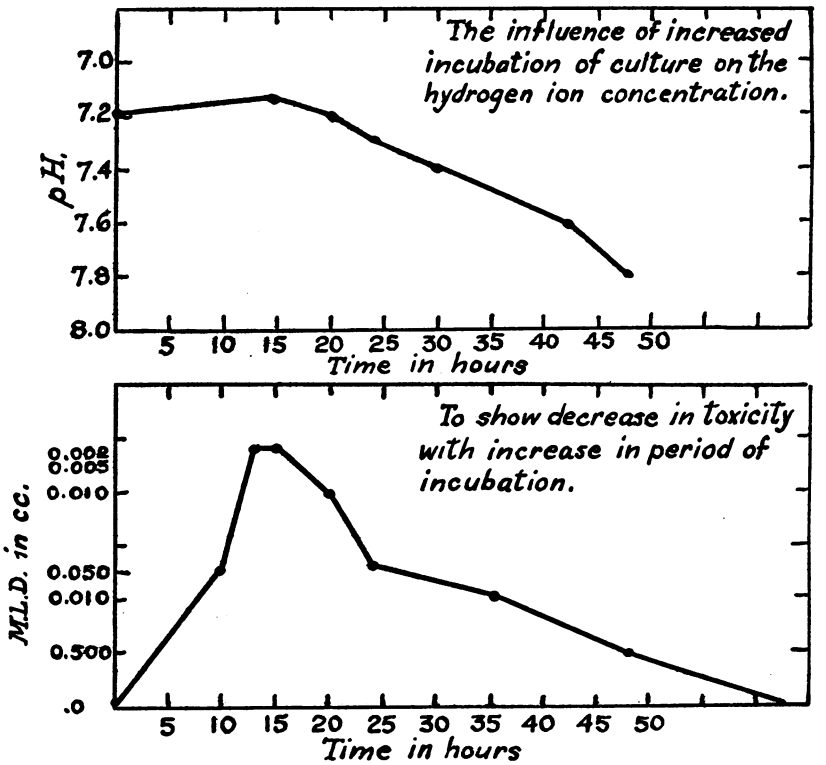

FIGURE 1.—Relation of alkalinity and toxicity to incubation period

fermented) was used. Two percent glucose was added to one lot of broth, 1 percent to another, and some was left sugar free. All were enriched with 5 percent horse serum. The flasks were inoculated and incubated for 15 hours, then filtered and the M. L. D. of the toxins was determined. The results are given in table 1. The broth containing the 2 percent glucose gave the strongest toxin. All however, were relatively weak, as the broth did not provide a suitable medium for the growth of *Cl. histolyticum*. This experiment was therefore repeated with ordinary meat infusion peptone broth (Parke-Davis) with and without glucose. Here again the broth

containing the 2 percent glucose gave the strongest toxin. This is shown in table 2.

TABLE 1.—*The effect of adding glucose to sugar-free broth (coli-fermented) on the toxin production by Cl. histolyticum*

Filtrate from 15-hour cultures	Amount of toxin	Number of mice used	Number of deaths
	Cc		
Sugar-free broth plus 5 percent horse serum	0.5	6	5
	.1	6	0
	.05	6	0
	.01	6	0
Sugar-free broth plus 5 percent horse serum plus 1 percent glucose	.5	6	6
	.1	6	3
	.05	6	0
	.01	6	0
Sugar-free broth plus 5 percent horse serum plus 2 percent glucose	.5	6	6
	.1	6	6
	.05	6	0
	.01	6	0

TABLE 2.—*The effect of adding glucose to nutrient broth used on the production of toxin by Cl. histolyticum*

Filtrate from 15-hour cultures	Amount of toxin used	Number of mice used	Number of deaths
	Cc		
Nutrient broth; no glucose	0.5	12	12
	.1	12	12
	.05	12	8
	.01	12	6
	.005	12	0
	.002	12	0
Nutrient broth plus 1 percent glucose	.5	12	12
	.1	12	12
	.05	12	11
	.01	12	6
	.005	12	0
	.002	12	0
Nutrient broth plus 2 percent glucose	.5	12	12
	.1	12	12
	.05	12	12
	.01	12	12
	.005	12	9
	.002	6	6

As an increase in acidity did not result after growing *Cl. histolyticum* in glucose broth, it was inferred that the glucose was utilized in some other manner. However, quantitative sugar determinations showed that there was no decrease in the amount of reducing substances present after a 15-hour growth of the culture. These determinations were made by the Shaffer-Hartman Cooper reduction method.

Since direct correlation between hemolytic activity and virulence is often encountered with many of the pathogenic bacteria, the possibility was considered that a hemolysin might account for the dif-

ferences in the toxicity of the glucose and glucose-free cultures of *Cl. histolyticum*. Weinberg and Seguin (*3*), also Hall (*4*), have shown that *Cl. histolyticum* does not hemolyze the red blood corpuscles of animal tissues. Mita (*1*), however, was able to demonstrate a hemolysin *in vitro* in liver broth cultures. In our work a hemolysin could not be demonstrated in the plain broth cultures, but a strong hemolysin was shown to be present in the 2 percent glucose broth cultures. It was necessary to use young cultures of 13 to 15 hours' growth in order to demonstrate a hemolysin, as it appears to be very unstable. The method proposed by Todd (*5*) for streptolysins was used. Table 3 gives the hemolysin titer obtained using varying amounts of culture against 0.5 cc of a 5 percent suspension of washed rabbit red blood corpuscles.

TABLE 3.—*Effect of glucose on the production of a hemolysin by C. histolyticum*

Amounts of culture used	13-hour 2-percent glucose broth culture	13-hour 1-percent glucose broth culture	13-hour plain broth culture; no glucose added
Cc			
0.4	++++	++++	+
0.35	++++	++++	+
0.3	++++	++++±	+
0.25	++++	++++±	±
0.2	++++	+++	±
0.16	++++	+++	±
0.14	++++	+++	±
0.12	++++	+++	±
0.1	++++	++	±
0.08	+++	++	−
0.06	++	+	−
0.04	+	+	−

Other reducing sugars such as maltose and galactose were found to give the same results as glucose. Nonreducing carbohydrates such as lactose and glycerine, however, did not stimulate hemolysin production.

It was considered that the presence of reducing sugars might stimulate the bacterial growth and thus account for the increased toxicity and for the presence of a hemolysin. Bacterial counts on the viable organisms, however, did not show this, as can be seen from table 4.

TABLE 4.—*Correlation between hemolysin production and the potency of the toxins in 13-hour cultures and its relationship to the number of viable organisms present*

Cultures	Hemolysins	M. L. D. of toxin	Number of bacteria
		Cc	*Cc*
Nutrient broth culture	Negative	0.05	3,000,000 per
2-percent glycerine nutrient broth	do	.05	4,000,000 per
2-percent galactose nutrient broth	4 plus with 0.1 cc	.01–.005	4,000,000 per
2-percent glucose nutrient broth	do	.005	3,000,000 per

Glucose also appears to favor proteolysis. This was not marked, but seems significant. Figure 2 illustrates the differences in digestion produced on milk agar plates by filtrates of cultures grown with and without glucose.

Reduced oxygen tension has been shown to favor certain types of proteolysis. Grossman, Dykerhoff, and Schoenebeck (*7*), also Waldschmidt-Leitz, Purr, and Ball (*8*), have shown that reduced glutathione acts as an activator of proteolytic enzymes of the cathepsin type. Voegtlin and Maver (*9*), in studying the *in vitro* autolysis of two malignant tumors, found that reduced oxygen tension activates tissue proteolysis and that it apparently operates through its influence on the sulphydryl system of the tissue.

Most hemolysins are known to be readily oxidized. Schwachman, Hellerman, and Barnett (*6*) have shown some of the ways by which the activity of pneumococcal hemolysin is controlled by oxidation and reduction. They demonstrated that the presence of sulphydryl groups could prevent its inactivation by preventing its oxidation.

It appears that the glucose in cultures of *Cl. histolyticum* may stimulate the production of a hemolysin and cause an increase in proteolysis, as shown on milk agar plates because of its reducing action. The effect of adding other reducing substances to the media was therefore tried.

Witte peptone, which is high in sulphydryl groups, was substituted for the Parke-Davis peptone; also, 0.1 percent cystine was added to the Parke-Davis peptone meat infusion broth. These were compared with the Parke-Davis meat infusion broths with and without glucose as to the strength of the toxins and hemolysins produced and for the presence of sulphydryl groups as shown by the sodium nitroprusside test. The results are given in table 5.

TABLE 5.—*A comparison of toxin production, etc., by Cl. histolyticum when grown in media of different reducing potentials*

Medium	M. L. D. of toxin	Hemolysin of red blood cells	Sodium nitroprusside test for sulphydryl groups
	Cc		
1 percent Witte meat infusion	0.002–0.005	++++	+++.
2 percent Witte meat infusion	0.002–0.005	++++	++++.
1 percent Parke-Davis meat infusion	0.01 –0.05	Negative	Negative.
2 percent Parke-Davis meat infusion	0.01 –0.05	Negative	+.
1 percent Parke-Davis meat infusion+0.1 percent cystine.	0.01 –0.05	Negative	+++++.
1 percent Parke-Davis meat infusion+2 percent glucose.	0.002–0.005	++++	Negative.

The Witte peptone meat infusion broth cultures were found to give the most potent toxins, having an M. L. D. of 0.002 cc to 0.005 cc for a 17–20-gram mouse. A strong hemolysin was also produced; 0.1 cc

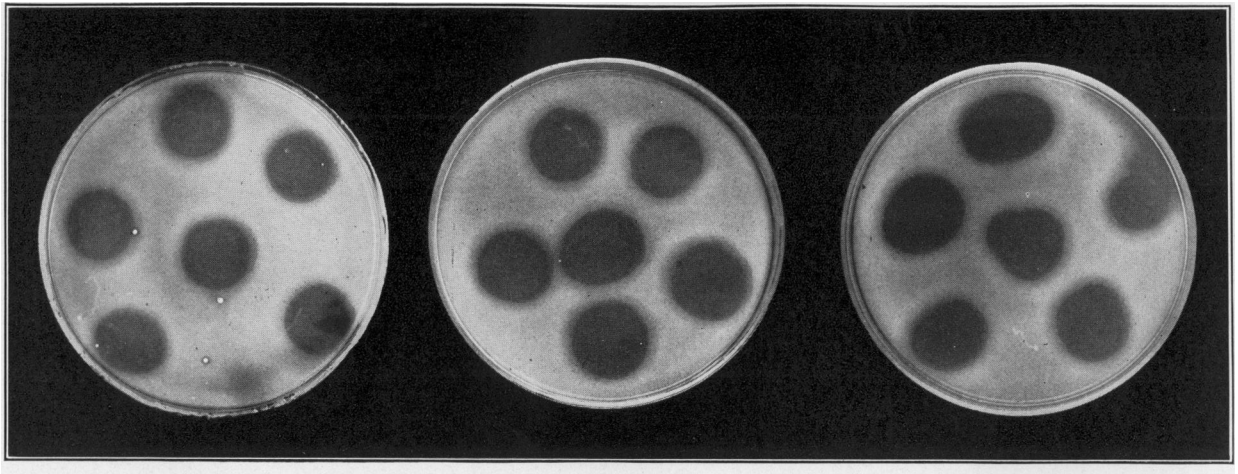

FIGURE 2.—Showing the difference in digestion produced on milk agar plates by filtrates of cultures of *Cl. histolyticum* grown with and without glucose.

of the cultures gave complete hemolysis of 0.5 cc of a 5 percent suspension of washed rabbit red blood corpuscles. The sodium nitroprusside test for the presence of sulphydryl groups was strongly positive. With the 1 percent Parke-Davis peptone meat infusion broth containing the 0.1 percent cystine a strong sodium nitroprusside test was also given, but here the hemolysin was entirely absent. The M. L. D. was also found to be much lower, varying from 0.01 cc to 0.05 cc for a 17–20-gram mouse. The Parke-Davis peptone meat infusion broth containing the 2 percent glucose gave a strong hemolysin and a much stronger toxin than the Parke-Davis peptone meat infusion broth without glucose. The M. L. D. varied between 0.002 cc and 0.005 cc, as compared with 0.01 cc to 0.05 cc for the broth cultures without glucose. The sulphydryl test was negative for both. No correlation was obtained between the toxicity (and hemolysins) and reduction of the media as shown by the presence of sulphydryl groups.

Estimations of the amount of reduction of the cultures in the different media were then made. Dyes (*10*) were used to measure the amount and the speed of reduction. These were used in the media in amounts that gave approximately the same color intensities for each dye. Small flasks or test tubes containing the media with the specific dye were heated in streaming steam for one hour to expel the free oxygen, cooled to about 40° C., and then inoculated with a young culture of *Cl. histolyticum*. These were sealed with a layer of vaseline and incubated at 37.5° C. Observations were made at 5-minute intervals, and the reduction of the different dyes was recorded. A buffer was not added to the media as there was no appreciable change in the pH of the cultures after 15 hours' incubation.

Results obtained are given in table 6.

TABLE 6.—*To show the rate of reduction of dyes by cultures of Cl. histolyticum grown on the different media*

Media pH 7.6	Methylene blue	Indigo carmine	Phenosafranine	Betaine viologen
1 percent Witte peptone meat infusion	30 minutes.	45 minutes.	5 hours.	15 hours.
1 percent Parke-Davis peptone meat infusion plus 2 percent glucose	15 minutes.	30 minutes.	5 hours.	Not reduced.
1 percent Parke-Davis peptone meat infusion	30 minutes.	45 minutes.	5 hours.	Do.

Although at the end of 15 hours' incubation no appreciable difference could be noted in the state of reduction between the Parke-Davis meat-infusion broth cultures with glucose and those without glucose, the rate of reduction was found to be more rapid in the glucose broth cultures. The Witte peptone-meat-infusion broth cultures, however, showed a much greater reduction at the end of the period of incuba-

tion. With betaine viologen as an indicator, about 20 percent reduction of the dye was observed.

From these investigations it appears that the production of a hemolysin and of a more potent toxin by the glucose broth cultures and the Witte peptone-meat-infusion broth cultures may be accounted for by the greater reducing power of these media.

PREPARATION OF TOXIN USED IN THE STANDARDIZATION OF HISTOLYTICUS ANTITOXIN [1]

A 1-percent Witte peptone-meat-infusion broth with a pH of 7.6 was used for the production of 60 liters of *histolyticus* toxin. Two-liter flasks were filled with sterile broth, heated one hour in streaming steam, and cooled to about 40° C. Each flask was inoculated with 10 cc of a 24-hour growth of the culture. The flasks were incubated at 37.5° C. for 15 hours. The cultures were then filtered through sterile paper pulp and then through Mandler filters. The toxin was precipitated from the 60 liters of filtrate with ammonium sulphate, using 750 grams per liter. The toxin formed a firm layer which was easily skimmed off. The precipitate was transferred to a Buchner funnel containing a layer of filter paper, and as much as possible of the fluid was removed by means of suction and the use of a dental rubber dam. The toxin was dried over phosphorus pentoxide and then ground thoroughly in a ball mill. The yield was 244 grams with an M. L. D. of 0.02 milligrams for a 17- to 20-gram mouse when inoculated intravenously.

THE STABILITY OF THE TOXIN UNDER DIFFERENT CONDITIONS OF EXPOSURE

Tests were made to determine the effects of variations of temperature and light on the toxin. Specimens of the dry toxin with a "test dose" of 0.9 mg were placed in dry, sterile ampuls, stoppered, and exposed to the following conditions:

1. To sunlight outside window.
2. At room temperature in the dark.
3. In warm room (37.5° C.) in the dark.
4. In cold room (4° to 5° C.) in vacuum jar.

After being exposed 1 month under the described conditions, the "test dose" of each was determined and found to be as follows:

	Mg.
1. Exposure to sunlight outside window	1.0
2. At room temperature in the dark room	1.0
3. In warm room (37.5° C.) in the dark	1.1
4. In cold room (4° to 5° C.) in vacuum jar	.9

[1] See preceding article on the standardization of *histolyticus* antitoxin.

SUMMARY

1. Meat infusion broth containing 1 percent Witte peptone is a suitable medium for the production of *Cl. histolyticum* toxin. Thirteen to fifteen hours' incubation at 37.5° C. was found to give maximum toxin production. The M. L. D. (intravenous in mice) varied between 0.002 and 0.005 cc. A 2 percent glucose meat infusion broth containing 1 percent Parke-Davis peptone was found to give a toxin of the same potency, but the results were not as regular.

2. Both the Witte peptone meat infusion broth cultures and the 2 percent glucose Parke-Davis peptone meat infusion broth cultures produced strong hemolysins as contrasted with the Parke-Davis meat infusion broth cultures without glucose, which were negative for hemolysins and which had a definitely lower M. L. D. The greater toxicity of the cultures containing glucose appears to be due to the reducing action of the glucose. The cultures containing Witte peptone showed the greatest amount of reduction after 15 hours' incubation.

3. The dried toxin appears to be quite stable. Little deterioration took place after exposure to sunlight and to a temperature of 37.5° C. for 30 days.

REFERENCES

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(2) Weinberg, M., and Randin, A.: Propriétés physico-chimiques du ferment fibrolytique d'origine microbienne. Compt. rendu de la Soc. Biol., **110**: 352 (1932).
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(8) Waldschmidt-Leitz, E., Purr, A., and Balls, A. K.: Naturwissenschaften, **18**: 644 (1930).
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(10) Clark, W. Mansfield.: The potential energies of oxidation-reduction systems and their biochemical significance. Medicine, **13**: 207 (1934).

PLAGUE FOUND IN PRAIRIE DOGS (CYNOMYS PARVIDENS) IN UTAH

Under date of August 26, 1936, Surgeon C. R. Eskey, of the United States Public Health Service plague laboratory in San Francisco, California, reported that plague had been demonstrated, by mass

inoculation of tissue material and cultures, in prairie dogs (*Cynomys parvidens*) shot on August 6, 1936, on a ranch 5 miles north-east of Panguitch, Garfield County, Utah. The report stated that cultures made on the usual media for differentiating *Pasteurella pestis* gave typical reactions for the plague organism. A guinea pig inoculated cutaneously from a blood agar plate was dead on the third day, and one inoculated subcutaneously from a plain agar culture was dead on the fourth day, demonstrating the high virulence of the material used. The macroscopic autopsy findings and microscopical examination of smears indicated a typical plague infection in both guinea pigs.

Previously plague infection had been demonstrated in fleas taken from 23 prairie dogs shot on a ranch 2 mile east of Hatch, in Garfield County; and a fatal epizootic among these animals had been reported in Utah and Montana.

It is believed that the finding of plague infection in fleas taken from prairie dogs was the first direct evidence that the disease existed in this animal in the United States, and that the subsequent report is the first record of plague being recovered from the tissues of prairie dogs in this country.

PUBLIC HEALTH SERVICE PUBLICATIONS

A List of Publications Issued During the Period January–June 1936

There is printed herewith a list of publications of the United States Public Health Service issued during the period January–June 1936.

The most important articles that appear each week in the PUBLIC HEALTH REPORTS are reprinted in pamphlet form, making possible a wider and more economical distribution of information that is of especial value and interest to public health workers and the general public.

All of the publications listed below except those marked with an asterisk (*) are available for free distribution and as long as the supply lasts may be obtained by addressing the Surgeon General, United States Public Health Service, Washington, D. C. Those publications marked with an asterisk are not available for free distribution but, unless stated to be "out of print", may be purchased from the Superintendent of Documents, Government Printing Office, Washington, D. C., *at the prices noted.* (No remittances should be sent to the Public Health Service.)

Periodicals

*Public Health Reports (weekly), January–June, vol. 51, nos. 1–26, pages 1 to 870. 5 cents a copy.

*Venereal Disease Information (monthly), January–June, vol. 17, nos. 1–6, pages 1 to 176. 5 cents a copy.

Reprints From the Public Health Reports

1725. The typhoid control program and results of 13 years' work in Williamson County, Tennessee, 1922–35. By W. C. Williams and E. L. Bishop. January 3, 1936. 15 pages.

1726. City smoke and its effects. A statement prepared for the congressional Subcommittee on Public Health, Hospitals, and Charities. January 3, 1936. 4 pages.

1727. Diets of low-income families surveyed in 1933. Health and depression studies no. 3. By Dorothy G. Wiehl. January 24, 1936. 21 pages.

1728. Calcium cyanide dust in ship fumigation. By C. L. Williams. February 7, 1936. 4 pages.

1729. Milk-sanitation status of urban communities. Urban communities in which pasteurized milk is both properly produced and properly pasteurized, and in which raw milk is at least properly produced, as shown by ratings of 90 percent or more reported by the State milk-sanitation authorities during the period January 1, 1934, to December 31, 1935. February 7, 1936. 4 pages.

1730. Results of field studies with the Brodie poliomyelitis vaccine. By A. G. Gilliam and R. H. Onstott. February 14, 1936. 12 pages.

1731. The place of mental hygiene in a Federal health program. By Walter L. Treadway. February 21, 1936. 13 pages.

1732. Prevention of experimental intranasal infection with certain neurotropic viruses by means of chemicals instilled into the nostrils. By Charles Armstrong and W. T. Harrison. February 28, 1936. 13 pages.

1733. Prevention of intravenously inoculated poliomyelitis of monkeys by intranasal instillation of picric acid. By Charles Armstrong. March 6, 1936. 3 pages.

1734. Biological products. Establishments licensed for the propagation and sale of viruses, serums, toxins, and analogous products. March 6, 1936. 6 pages.

1735. The official United States and international unit for standardizing gas gangrene antitoxin (oedematiens). By Ida A. Bengtson. March 13, 1936. 10 pages.

1736. Results of a dental examination of 1,908 white and colored males at the Ohio State Reformatory. By W. M. Gafafer and C. T. Messner. March 27, 1936. 12 pages.

1737. The picture of heart disease mortality obtained from vital statistics in Washington, D. C., during 1932. By O. F. Hedley. March 20, 1936. 14 pages.

1738. Changes in the incidence and fatality of smallpox in recent decades. By A. W. Hedrich. April 3, 1936. 30 pages.

1739. Acute response of guinea pigs to vapors of some new commercial organic compounds. IX. Pentanone (methyl propyl ketone). By W. P. Yant, F. A. Patty, and H. H. Schrenk. April 3, 1936. 8 pages.

1740. History and frequency of smallpox vaccinations and cases in 9,000 families. Based on Nation-wide periodic canvasses, 1928–31. By Selwyn D. Collins. April 17, 1936. 37 pages.

1741. Public Health Service publications. A list of publications issued during the period July–December 1935. April 17, 1936. 3 pages.

1742. An occupational dermatitis due to heat decomposition of dyes. By Louis Schwartz and C. D. Hocker. April 24, 1936. 17 pages.

1743. Mortality in certain States during 1935 with comparative data for recent years. May 1, 1936. 10 pages.

1744. The significance of infant mortality rates. By Mayhew Derryberry and Edgar Van Buskirk. May 1, 1936. 7 pages.

1745. A comparative study of certain characteristics of 1,000 inmates of the Northeastern Penitentiary. I. Age. By Barkev S. Sanders. May 8, 1936. 21 pages.

1746. Studies of sewage purification. IV. The use of chlorine for the correction of sludge bulking in the activated sludge process. By Russell S. Smith and W. C. Purdy. May 15, 1936. 7 pages.

1747. Acute response of guinea pigs to vapors of some new commercial organic compounds. X. Hexanone (methyl butyl ketone). By H. H. Schrenk, W. P. Yant, and F. A. Patty. May 15, 1936. 8 pages.

1748. Sickness among male industrial employees during the final quarter of 1935 and the entire year. By Dean K. Brundage. May 22, 1936. 3 pages.

1749. Engineering control of occupational diseases. By J. J. Bloomfield. May 22, 1936. 13 pages.

1750. The preparation of a concentrate of vitamins B_1 and B_2 from brewers' yeast. By Maurice I. Smith and Atherton Seidell. May 29, 1936. 4 pages.

1751. Application of the preliminary sanitary survey to flooded areas. By J. M. DallaValle and J. J. Bloomfield. May 29, 1936. 6 pages.

1752. Rat-proof construction and its effect on the control of rat life on ships. Instances of permanent and apparent automatic control effected by this type of construction observed on 50 ships at the port of New York. By B. E. Holsendorf. May 29, 1936. 13 pages.

1753. Smallpox immunity in 5,000 college students. By R. C. Bull and S. L. Rankin. June 5, 1936. 13 pages.

1754. The development of a technique for measuring the knowledge and practice of midwives. By Mayhew Derryberry and Josephine Daniel. June 12, 1936. 15 pages.

1755. Marine hospitals and beneficiaries of the Public Health Service. By S. L. Christian. June 19, 1936. 13 pages; 3 plates.

1756. Acute response of guinea pigs to vapors of some new commercial organic compounds. XI. Secondary amyl acetate. By F. A. Patty, W. P. Yant, and H. H. Schrenk. June 19, 1936. 9 pages.

1757. Relation of physical defects to the physical growth of children of 21 States. Physical measurement studies no. 3. By William M. Gafafer. June 26, 1936. 11 pages.

Public Health Bulletins

222. History of county health organizations in the United States 1908–33. Compilation by John A. Ferrell and Pauline A. Mead. March 1936. 469 pages.

223. Observations on Indian health problems and facilities. By Joseph W. Mountin and J. G. Townsend. February 1936. 47 pages.

224. Atmospheric pollution of American cities for the years 1931 to 1933. With special reference to the solid constituents of the pollution. By James E. Ives, Rollo H. Britten, David W. Armstrong, W. A. Gill, and Frederick H. Goldman. March 1936. 75 pages; 1 plate.

225. Some features of tuberculosis mortality distribution in the United States. By L. L. Lumsden and C. C. Dauer. March 1936. 39 pages.

226. Dental survey of school children, ages 6–14 years made in 1933–34 in 26 States. By C. T. Messner, W. M. Gafafer, F. C. Cady, and H. T. Dean. May 1936. 248 pages.

227. A survey of dental activities of State departments and institutions of the United States. By F. C. Cady, H. T. Dean, and C. T. Messner. June 1936. 217 pages.

National Institute of Health Bulletin

166. Epidemic amoebic dysentery. The Chicago outbreak of 1933. By Herman N. Bundesen, Joel I. Connolly, Isaac D. Rawlings, Arthur E. Gorman, George W. McCoy, and Albert V. Hardy. March 1936. 187 pages.

Annual Report

*Annual Report of the Surgeon General of the United States Public Health Service for the fiscal year 1935. 158 pages. 75 cents.

Unnumbered Publications

Index to Public Health Reports, vol. 50, part 2 (July–December 1935). 1936. 22 pages.

*National Negro Health Week program. This pamphlet is published annually, usually about the middle of March, for community leaders in an effort to suggest ways and means by which interested individuals and organizations may be organized for a concerted and effective attack upon the community's disease problems. Twenty-second annual observance. 1936. 8 page folder.

*National Negro Health Week poster. Twenty-second annual observance. 1936.

*National Negro Health Week leaflet. Twenty-second annual observance. 1936. 2 pages.

Reprints from Venereal Disease Information

53. Syphilis Control in New York State. By Thomas Parran. Vol. 16, No. 9. 6 pages.

Supplements to Venereal Disease Information

1. The evaluation of serodiagnostic tests for syphilis in the United States. Detailed report of results. By H. S. Cumming, H. H. Hazen, Arthur H. Sanford, F. E. Senear, Walter M. Simpson, and R. A. Vonderlehr. 49 pages.

Venereal Disease Bulletin

89. Facts about syphilis, gonorrhea, and other venereal diseases. 33 pages.

DEATHS DURING WEEK ENDED AUG. 22, 1936

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

	Week ended Aug. 22, 1936	Corresponding week, 1935
Data from 86 large cities of the United States:		
Total deaths	7,368	7,073
Deaths per 1,000 population, annual basis	10.3	9.9
Deaths under 1 year of age	470	499
Deaths under 1 year of age per 1,000 estimated live births	42	46
Deaths per 1,000 population, annual basis, first 34 weeks of year	12.6	11.7
Data from industrial insurance companies:		
Policies in force	68,265,792	67,486,280
Number of death claims	11,329	10,830
Death claims per 1,000 policies in force, annual rate	8.7	8.4
Death claims per 1,000 policies, first 34 weeks of year, annual rate	10.3	10.0

PREVALENCE OF DISEASE

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

UNITED STATES

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 August 29, 1936, and August 31, 1935

Cases of certain communicable diseases reported by telegraph by State health officers for weeks ended Aug. 29, 1936, and Aug. 31, 1935

Division and State	Diphtheria		Influenza		Measles		Meningococcus meningitis	
	Week ended Aug. 29, 1936	Week ended Aug. 31, 1935	Week ended Aug. 29, 1936	Week ended Aug. 31, 1935	Week ended Aug. 29, 1936	Week ended Aug. 31, 1935	Week ended Aug. 29, 1936	Week ended Aug. 31, 1935
New England States:								
Maine	1		1		21	9	0	0
New Hampshire							0	0
Vermont	1					6	0	0
Massachusetts	3	4			27	21	1	1
Rhode Island					1	5	0	0
Connecticut	1	2	1		3	5	1	3
Middle Atlantic States:								
New York	12	19	[1] 2	[1] 6	75	127	9	14
New Jersey	6	4	6	1	26	14	2	3
Pennsylvania	17	29			47	83	4	4
East North Central States:								
Ohio	17	9	8	34	13	27	1	3
Indiana	5	16	4	26	3	2	2	0
Illinois [2]	25	22	2	4	11	15	2	5
Michigan	3			2	14	27	3	0
Wisconsin		6	12	16	16	63	1	1
West North Central States:								
Minnesota	2	1	3	1	4	2	0	2
Iowa	5	5	1	1		2	1	0
Missouri	10	25	9	18		6	2	2
North Dakota	4		1		1	1	0	1
South Dakota		1			1		1	0
Nebraska	5	2			3	6	0	3
Kansas	5	1			2	8	0	1
South Atlantic States:								
Delaware [3]		1				2	0	2
Maryland [3]	3	3		1	6	9	3	3
District of Columbia		8			4		1	2
Virginia [3]	22	24			16	1	2	3
West Virginia	11	22	9	51	2	17	1	3
North Carolina [3]	36	36	5	4	6		2	2
South Carolina [4]	4	8	53	51	6		1	0
Georgia [4]	12	16					1	2
Florida [4]	1	19	1		1	1	3	0

See footnotes at end of table.

Cases of certain communicable diseases reported by telegraph by State health officers for weeks ended Aug. 29, 1936, and Aug. 31, 1935—Continued

Division and State	Diphtheria		Influenza		Measles		Meningococcus meningitis	
	Week ended Aug. 29, 1936	Week ended Aug. 31, 1935	Week ended Aug. 29, 1936	Week ended Aug. 31, 1935	Week ended Aug. 29, 1936	Week ended Aug. 31, 1935	Week ended Aug. 29, 1936	Week ended Aug. 31, 1935
East South Central States:								
Kentucky	11	38	12	3	15	9	3	2
Tennessee	17	24	7	2		1	2	0
Alabama [4]	26	21	1	39	4	13	2	0
Mississippi [3]	13	21					0	0
West South Central States:								
Arkansas	4	12	3	1			0	0
Louisiana [4]	9	24	23	20		8	2	1
Oklahoma [5]	6	8	8	7	3		0	0
Texas [4]	28	58	8	12	18	29	0	0
Mountain States:								
Montana [2]	1	1		1		4	1	0
Idaho					1		0	0
Wyoming						11	0	0
Colorado	3	9				1	0	0
New Mexico	5	1			1		0	0
Arizona	2	2	17	6	16	1	2	0
Utah [3]	1				3		0	0
Pacific States:								
Washington		1			4	5	0	2
Oregon [2]	1	2	4		4	69	2	1
California	24	24	14	3	43	82	1	5
Total	362	529	215	310	421	692	59	71
First 35 weeks of year	15,802	19,098	142,123	104,679	270,969	696,904	6,068	4,292

Division and State	Poliomyelitis		Scarlet fever		Smallpox		Typhoid fever	
	Week ended Aug. 29, 1936	Week ended Aug. 31, 1935	Week ended Aug. 29, 1936	Week ended Aug. 31, 1935	Week ended Aug. 29, 1936	Week ended Aug. 31, 1935	Week ended Aug. 29, 1936	Week ended Aug. 31, 1935
New England States:								
Maine	1	16	7	13	0	0	3	4
New Hampshire	0	6		2	0	0	0	1
Vermont	0	2	4	0	0	0	0	0
Massachusetts	3	166	26	38	0	0	4	2
Rhode Island	0	58	11	1	0	0	0	0
Connecticut	0	39	3	13	0	0	4	3
Middle Atlantic States:								
New York	10	460	83	80	0	0	41	29
New Jersey	2	35	16	10	0	0	11	3
Pennsylvania	6	13	59	65	0	0	24	23
East North Central States:								
Ohio	14	14	69	49	1	0	28	49
Indiana	1	2	11	29	0	0	20	18
Illinois [2]	19	19	82	93	3	0	25	28
Michigan	3	108	51	33	0	7	5	18
Wisconsin	1	4	69	55	0	1	1	1
West North Central States:								
Minnesota	2	5	18	35	0	0	2	8
Iowa	2	4	10	25	2	0	8	3
Missouri	1	0	19	19	2	0	27	19
North Dakota	0	1	3	4	0	0	0	1
South Dakota	0	0	4	6	0	3	0	2
Nebraska	0	0	5	2	1	2	0	0
Kansas	1	2	17	10	1	1	19	15
South Atlantic States:								
Delaware [3]	1	2		5	0	0	0	7
Maryland [3]	0	5	11	17	0	0	9	26
District of Columbia	1	5		4	0	0	0	5
Virginia [2]	5	31	12	23	0	0	19	36
West Virginia	1	3	12	47	0	0	9	18
North Carolina [2]	0	9	24	25	0	0	26	19
South Carolina [4]	1	1		3	0	1	18	24
Georgia [4]	10	0	10	6	0	0	38	45
Florida [4]	4	0	4	3	0	0	2	0

See footnotes at end of table.

Cases of certain communicable diseases reported by telegraph by State health officers for weeks ended Aug. 29, 1936, and Aug. 31, 1935—Continued

Division and State	Poliomyelitis		Scarlet fever		Smallpox		Typhoid fever	
	Week ended Aug. 29, 1936	Week ended Aug. 31, 1935	Week ended Aug. 29, 1936	Week ended Aug. 31, 1935	Week ended Aug. 29, 1936	Week ended Aug. 31, 1935	Week ended Aug. 29, 1936	Week ended Aug. 31, 1935
East South Central States:								
Kentucky	7	36	13	40	0	0	56	70
Tennessee	19	1	8	16	0	0	33	36
Alabama [4]	16	4	13	11	0	0	36	16
Mississippi [3]	15	6	8	14	0	0	9	9
West South Central States:								
Arkansas	0	0	2	8	0	0	12	9
Louisiana [4]	0	1	7	10	0	0	23	19
Oklahoma [5]	0	0	7	4	0	0	16	41
Texas [4]	1	9	15	21	0	4	43	59
Mountain States:								
Montana [3]	0	0	4	5	8	0	5	7
Idaho	0	0	1	1	0	0	2	2
Wyoming	0	0	4	6	1	0	1	0
Colorado	2	0	6	11	0	0	1	6
New Mexico	1	0	9	4	0	0	13	14
Arizona	0	1	1	1	0	0	4	2
Utah [3]	0	0	10	14	2	0	6	2
Pacific States:								
Washington	2	1	15	9	0	3	3	4
Oregon [3]	0	1	16	16	0	0	2	8
California	12	24	65	49	0	5	12	11
Total	164	1,088	844	955	21	27	614	721
First 35 weeks of year	1,664	5,417	185,600	182,211	6,317	5,368	8,081	10,718

[1] New York City only.
[2] Rocky Mountain spotted fever, week ended Aug. 29, 1936, 10 cases, as follows: Illinois, 1; Delaware, 2; Virginia, 2; North Carolina, 1; Montana, 3; Oregon 1.
[3] Week ended earlier than Saturday.
[4] Typhus fever, week ended Aug. 29, 1936, 73 cases, as follows: South Carolina, 1; Georgia, 38; Florida, 1; Alabama, 24; Louisiana, 3; Texas, 6.
[5] 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
June 1936										
Missouri	13	73	99	164	66	-------	1	348	22	46
July 1936										
Arizona	4	8	49	7	197	-------	1	19	0	17
Massachusetts	6	38	-------	1	1,283	2	4	257	0	49
Missouri	8	39	61	201	68	2	3	157	25	62
Montana	2	1	3	-------	11	-------	2	75	62	12
New York	39	136	-------	6	3,664	-------	19	739	0	47
Oregon	1	2	17	4	40	-------	-------	39	5	17
South Dakota	2	4	11	-------	9	-------	1	38	14	3
Vermont	-------	-------	-------	-------	69	-------	5	15	0	5
Virginia	14	19	63	43	215	23	3	45	0	50
Washington	1	1	5	-------	217	-------	10	48	6	15

Summary of Monthly Reports from States—Continued

June 1936

	Cases
Missouri:	
Chicken pox	78
Dysentery	41
Epidemic encephalitis	1
Mumps	115
Ophthalmia neonatorum	2
Rabies (in animals)	16
Septic sore throat	33
Trachoma	58
Tularaemia	2
Undulant fever	4
Whooping cough	76

July 1936

	Cases
Anthrax:	
Arizona	1
Massachusetts	1
New York	1
Chicken pox:	
Arizona	14
Massachusetts	312
Missouri	43
Montana	85
New York	814
Oregon	28
South Dakota	2
Vermont	24
Virginia	33
Washington	106
Dysentery:	
Arizona	27
Missouri	110
New York (amoebic)	3
New York (bacillary)	23
Virginia (diarrhea included)	515
Epidemic encephalitis:	
Arizona	1
New York	0
Oregon	2
South Dakota	1
Washington	4

July 1936—Continued

	Cases
German measles:	
Arizona	24
New York	175
Vermont	12
Washington	36
Impetigo contagiosa:	
Oregon	11
Mumps:	
Arizona	107
Massachusetts	446
Missouri	63
Montana	74
Oregon	16
South Dakota	16
Vermont	45
Virginia	62
Washington	56
Ophthalmia neonatorum:	
Missouri	1
New York	8
Paratyphoid fever:	
New York	7
Virginia	2
Washington	1
Puerperal septicemia:	
Montana	1
Rabies in animals:	
Massachusetts	16
Missouri	12
New York [1]	1
Oregon	2
Washington	3
Rabies (man):	
New York	1
Rocky Mountain spotted fever:	
Montana	5
Oregon	2
Virginia	13
Washington	1
Scabies:	
Oregon	4
Washington	1

July 1936—Continued

	Cases
Septic sore throat:	
Massachusetts	7
Missouri	22
New York	44
Oregon	2
Washington	3
Tetanus:	
New York	10
Trachoma:	
Arizona	30
Missouri	42
Montana	1
Trichinosis:	
New York	1
Tularemia:	
Virginia	3
Typhus fever:	
New York	3
Undulant fever:	
Arizona	8
Massachusetts	3
Missouri	2
Montana	1
New York	25
Oregon	1
South Dakota	1
Vermont	2
Virginia	1
Washington	2
Vincent's infection:	
New York [1]	36
Oregon	5
Whooping cough:	
Arizona	49
Massachusetts	467
Missouri	90
Montana	53
New York	1,201
Oregon	145
South Dakota	1
Vermont	34
Virginia	189
Washington	98

[1] Exclusive of New York City.

PLAGUE IN PRAIRIE DOGS IN GARFIELD COUNTY, UTAH

Under date of August 24, 1936, plague infection was reported in fleas taken from 23 prairie dogs, *Cynomys parvidens*, shot on a ranch 2 miles east of Hatch, Garfield County, Utah. Plague infection was reported, under date of August 26, 1936, to have been proved by mass inoculation of material from 2 prairie dogs shot August 6 on a ranch 5 miles northeast of Panguitch, Garfield County, Utah. See page 1279.

WEEKLY REPORTS FROM CITIES

City reports for week ended Aug. 22, 1936

This table summarizes the reports received weekly from a selected list of 140 cities for the purpose of showing a cross section of the current urban incidence of the communicable diseases listed in the table. Weekly reports are received from about 700 cities, from which the data are tabulated and filed for reference.

State and city	Diphtheria cases	Influenza		Measles cases	Pneumonia deaths	Scarlet fever cases	Smallpox cases	Tuberculosis deaths	Typhoid fever cases	Whooping cough cases	Deaths, all causes
		Cases	Deaths								
Maine:											
Portland	0	------	0	0	1	0	0	0	0	3	15
New Hampshire:											
Concord	0	------	0	0	0	0	0	0	0	0	10
Nashua	0	------	--------	0	-------	0	0	-------	0	0	--------
Vermont:											
Barre	-------	------	--------	-------	-------	-------	-------	-------	-------	-------	--------
Burlington	0	------	0	0	0	0	0	0	0	0	6
Rutland	0	------	0	0	0	0	0	0	0	2	6
Massachusetts:											
Boston	1	------	0	8	5	13	0	10	0	63	187
Fall River	0	------	0	0	0	1	0	2	0	0	23
Springfield	0	------	0	0	0	1	0	1	0	3	32
Worcester	0	------	0	1	3	3	0	1	0	9	39
Rhode Island:											
Pawtucket	0	------	0	0	0	0	0	0	0	0	--------
Providence	1	------	0	0	1	4	0	1	0	27	35
Connecticut:											
Bridgeport	0	------	0	4	0	1	0	1	0	2	21
Hartford	0	------	0	1	2	1	0	0	1	2	30
New Haven	0	------	0	0	1	1	0	0	0	13	23
New York:											
Buffalo	1	------	0	6	0	6	0	8	0	15	141
New York	16	2	1	41	64	18	0	106	12	135	1,284
Rochester	0	------	0	1	1	1	0	3	1	2	60
Syracuse	0	------	1	1	2	2	0	1	0	7	50
New Jersey:											
Camden	1	------	0	5	1	0	0	0	0	1	22
Newark	0	------	0	6	7	3	0	5	0	30	86
Trenton	1	------	0	0	2	2	0	1	3	3	31
Pennsylvania:											
Philadelphia	0	------	0	2	10	8	0	27	10	90	369
Pittsburgh	1	2	0	1	11	11	0	6	1	0	122
Reading	0	------	0	2	0	1	0	2	2	5	27
Ohio:											
Cincinnati	4	------	0	2	4	3	0	10	0	2	129
Cleveland	6	------	0	2	11	13	0	11	0	97	183
Columbus	1	------	0	1	2	2	0	4	0	17	79
Toledo	0	------	0	0	1	-------	-------	3	1	24	70
Indiana:											
Anderson	0	------	0	0	0	1	0	0	0	6	9
Fort Wayne	0	------	0	0	3	0	0	1	0	1	21
Indianapolis	1	------	1	3	7	4	0	3	1	3	107
South Bend	0	------	0	0	2	0	0	0	2	1	14
Terre Haute	0	------	0	0	0	0	0	0	0	0	25
Illinois:											
Alton	0	------	0	0	0	0	0	0	0	0	7
Chicago	7	------	4	6	21	30	0	30	8	106	587
Elgin	2	------	0	0	0	0	0	0	0	5	10
Moline	0	------	0	0	0	0	0	1	1	1	5
Springfield	0	------	0	2	1	3	0	0	0	1	12
Michigan:											
Detroit	3	------	0	2	4	17	0	18	5	97	257
Flint	0	------	0	0	1	4	0	1	0	3	25
Grand Rapids	0	------	0	3	0	0	0	0	0	16	27
Wisconsin:											
Kenosha	0	------	0	0	0	1	0	0	0	0	6
Madison	0	------	0	0	0	0	0	0	0	16	3
Milwaukee	0	------	0	6	1	7	0	2	0	50	64
Racine	-------	------	-------	-------	-------	-------	-------	-------	-------	-------	--------
Superior	0	------	0	0	0	1	0	0	0	0	9
Minnesota:											
Duluth	0	------	0	0	3	0	6	0	0	8	23
Minneapolis	1	------	0	0	3	2	0	0	0	2	80
St. Paul	0	------	0	0	3	1	0	2	0	9	57
Iowa:											
Cedar Rapids	0	------	--------	0	-------	0	0	-------	0	1	--------
Davenport	1	------	--------	0	-------	0	0	-------	0	0	--------
Des Moines	0	------	--------	0	1	0	0	-------	0	0	23
Sioux City	0	------	--------	0	-------	0	0	-------	0	0	--------
Waterloo	0	------	--------	0	-------	1	0	-------	0	3	--------

City reports for week ended Aug. 22, 1936—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								
Missouri:											
Kansas City	0		1	0	4	2	0	3	0	2	120
St. Joseph											
St. Louis	5		0	1	1	8	0	10	7	14	216
North Dakota:											
Fargo	0		0	0	0	3	0	0	0	0	7
Grand Forks	0			0		0	0		0	0	
Minot	0		0	0	0	1	0	0	0	0	6
South Dakota:											
Aberdeen	0			0		0	0		0	0	
Sioux Falls	0		0	0	0	2	0	0	0	0	7
Nebraska:											
Omaha	1		0	0	3	1	0	0	0	0	43
Kansas:											
Lawrence	0		0	0	1	0	0	0	0	0	12
Topeka	0		0	0	3	0	0	0	0	0	22
Wichita	0		0	1	1	1	0	1	1	1	29
Delaware:											
Wilmington											
Maryland:											
Baltimore	4		0	8	10	6	0	6	0	100	176
Cumberland	0			0	0	0	0		0	0	
Frederick	0		0	0	0	0	0	0	0	0	
District of Columbia:											
Washington	2		0	3	3	2	0	6	3	25	147
Virginia:											
Lynchburg	2		0	0	0	0	0	0	2	4	11
Richmond	0		0	0	3	0	0	3	1	0	46
Roanoke	0		0	0	0	0	0	0	0	0	13
West Virginia:											
Charleston	0		0	1	1	0	0	0	0	0	14
Huntington	2			0		0	0		0	0	
Wheeling	1		0	0	1	0	0	0	0	2	18
North Carolina:											
Gastonia	0			0		0	0		0	0	
Raleigh	0		0	0	1	0	0	0	0	0	15
Wilmington	0		0	0	0	0	0	0	0	0	13
Winston-Salem	0		0	0	0	0	0	1	0	0	16
South Carolina:											
Charleston	1		0	0	0	0	0	1	0	0	21
Columbia											
Florence	0		0	0	0	0	0	1	0	0	12
Greenville	0		0	0	1	0	0	0	0	0	5
Georgia:											
Atlanta	1	1	0	5	5	2	0	6	2	0	106
Brunswick	0		0	0	0	0	0	1	0	0	3
Savannah	3		0	0	0	0	0	1	2	0	30
Florida:											
Miami	0		0	1	1	0	0	0	0	0	21
Tampa	1		0	0	1	2	0	0	0	0	19
Kentucky:											
Ashland	0			0	1	0	0		0	0	16
Covington	0		0	0	0	0	0	1	0	0	1
Lexington	0		0	0	0	1	0	0	0	0	18
Louisville	1		0		3	1	0	3	2	1	83
Tennessee:											
Knoxville	2		0	0	2	0	0	0	0	0	20
Memphis	1		0	0	2	3	0	7	1	8	111
Nashville	3		0	0	4	0	0	3	2		60
Alabama:											
Birmingham	0		0	0	11	1	0	2	2	0	83
Mobile	1		0	0	1	0	0	1	0	0	12
Montgomery	0			0		0	0		1	0	
Arkansas:											
Fort Smith											
Little Rock	1		1	0	2	0	0	3	1	0	6
Louisiana:											
Lake Charles	0		0	0	0	0	0	1	0	0	6
New Orleans	1	2	2	2	7	0	0	10	7	6	138
Shreveport	0		0	0	7	0	0	2	0	0	48
Oklahoma:											
Oklahoma City	1		0	0	1	1	0	1	4	0	42
Tulsa	0			0		0	0		2	0	

City reports for week ended Aug. 22, 1936—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								
Texas:											
Dallas	5	1	1	2	0	3	0	3	2	0	75
Fort Worth	1	------	0	0	3	1	0	1	0	0	47
Galveston	0	------	0	0	2	0	0	1	1	0	14
Houston	0	------	0	0	6	0	0	3	0	0	79
San Antonio	0	------	0	0	5	0	0	6	0	2	80
Montana:											
Billings	0	------	0	0	0	1	0	0	0	1	9
Great Falls	0	------	0	0	2	2	0	0	0	0	5
Helena	0	------	0	0	0	0	0	0	0	0	2
Missoula	0	------	0	0	0	0	0	0	0	0	8
Idaho:											
Boise	0	------	0	0	0	0	0	1	0	0	9
Colorado:											
Colorado Springs	0	------	0	0	0	3	0	2	0	1	12
Denver	1	------	0	2	4	4	0	6	2	32	70
Pueblo	0	------	0	0	0	2	0	0	1	0	5
New Mexico:											
Albuquerque	0	------	0	0	0	0	0	4	0	0	11
Utah:											
Salt Lake City	0	------	0	1	0	2	1	0	0	8	23
Washington:											
Seattle	0	------	0	0	0	1	0	6	1	8	74
Spokane	0	------	0	------	2	6	0	0	0	0	19
Tacoma	0	------	0	0	1	0	0	2	0	0	20
Oregon:											
Portland	0	------	0	4	4	2	0	4	1	5	65
Salem	0	3	------	0	------	0	0	------	0	0	------
California:											
Los Angeles	9	7	1	18	12	4	0	16	0	36	266
Sacramento	0	------	0	0	1	7	0	1	0	23	15
San Francisco	2	------	2	3	8	5	0	4	0	19	146

State and city	Meningococcus meningitis		Poliomyelitis cases	State and city	Meningococcus meningitis		Poliomyelitis cases
	Cases	Deaths			Cases	Deaths	
Massachusetts:				District of Columbia:			
Boston	1	0	1	Washington	1	0	0
New York:				Virginia:			
New York	3	0	1	Lynchburg	0	0	2
Syracuse	0	0	1	Kentucky:			
New Jersey:				Ashland	0	1	0
Trenton	0	0	1	Louisville	1	1	0
Pennsylvania:				Tennessee:			
Pittsburgh	1	0	0	Memphis	2	0	2
Ohio:				Alabama:			
Toledo	0	0	1	Birmingham	0	0	5
Indiana:				Mobile	0	0	1
Indianapolis	1	1	0	Louisiana:			
Illinois:				New Orleans	1	0	1
Alton	0	0	1	Oklahoma:			
Chicago	3	0	6	Oklahoma City	1	0	1
Michigan:				Texas:			
Detroit	1	1	0	Dallas	0	0	2
Grand Rapids	0	0	1	Colorado:			
Wisconsin:				Denver	0	0	1
Madison	1	0	0	Washington:			
Minnesota:				Spokane	0	0	2
Minneapolis	2	0	0	Oregon:			
Iowa:				Portland	1	0	0
Des Moines	1	0	0	California:			
Missouri:				Los Angeles	1	0	1
St. Louis	0	0	1	San Francisco	0	1	0
Maryland:							
Baltimore	1	1	0				

Epidemic encephalitis.—Cases: New York, 1; Detroit, 2; Wichita, 1; Denver, 1.
Pellagra.—Cases: Atlanta, 3; Savannah, 1; Dallas, 1; Los Angeles, 1.
Typhus fever.—Cases: New York, 1; Atlanta, 4; Montgomery, 1.

FOREIGN AND INSULAR

BRITISH INDIA

Vital statistics—Fourth quarter, ended December 31, 1935. The following table shows the births and deaths reported in British India during the fourth quarter, ended December 31, 1935, together with the number of deaths reported from certain diseases.

Population	279,982,981
Births	2,818,217
Births per 1,000 population	40
Deaths	1,732,752
Deaths per 1,000 population	25

Deaths from:	
Cholera	42,015
Diarrhea and dysentery	70,288
Fevers	1,007,841
Plague	2,276
Respiratory diseases	123,027
Smallpox	9,502

CANADA

Manitoba—Bois Sevain—Poliomyelitis.—From July 25 to August 10, 1936, 25 new cases of poliomyelitis were reported in the Bois Sevain district, southwestern Manitoba, Canada. A previous report stated that up to July 24, 11 cases of poliomyelitis had been reported in the same district, making a total of 36 cases of poliomyelitis reported to August 10, 1936.

Provinces—Communicable diseases—2 weeks ended August 8, 1936.—During the 2 weeks ended August 8, 1936, cases of certain communicable diseases were reported by the Department of Pensions and National Health of Canada as follows:

Disease	Prince Edward Island	Nova Scotia	New Brunswick	Quebec	Ontario	Manitoba	Saskatchewan	Alberta	British Columbia	Total
Cerebrospinal meningitis				1	1	1				3
Chicken pox		1	1	60	85	14	19	32	23	235
Diphtheria		7	22	44	8	9	1		1	92
Dysentery				1	10		1		1	13
Erysipelas				7	3	2		1	3	16
Influenza		20			5				5	30
Leprosy				1						1
Lethargic encephalitis						1				1
Measles		2	3	150	273	61	57	25	41	612
Mumps					125	4	10	4	30	173
Paratyphoid fever					2			1		3
Pneumonia	1				17				2	20
Poliomyelitis				3	3	17	2		2	27
Scarlet fever		7	5	88	136	55	9	50	6	356
Smallpox							1			1
Trachoma									2	2
Tuberculosis	4	43	49	111	53	52	27	2	23	364
Typhoid fever			5	35	12	2	16	2	1	73
Undulant fever				1	4					5
Whooping cough		8		196	229	16	12	10	48	519

YUGOSLAVIA

Communicable diseases—July 1936.—During the month of July 1936, certain communicable diseases were reported in Yugoslavia as follows:

Disease	Cases	Deaths	Disease	Cases	Deaths
Anthrax	138	9	Poliomyelitis	32	5
Cerebrospinal meningitis	9	4	Scarlet fever	271	3
Diphtheria and croup	483	39	Sepsis	6	1
Dysentery	504	54	Tetanus	52	18
Erysipelas	213	13	Typhoid fever	556	38
Measles	145	----------	Typhus fever	53	2
Paratyphoid fever	10	1			

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 August 28, 1936, pages 1214–1227. A similar cumulative table will appear in the PUBLIC HEALTH REPORTS to be issued September 25, 1936, and thereafter, at least for the time being, in the issue published on the last Friday of each month.

Plague

Algeria—Philippeville.—On August 22, 1936, 1 suspected case of plague was reported in Philippeville, Algeria.

Brazil—Sañtos.—Three cases of plague with 1 death during the week ended August 8, 1936, have been reported at Santos, Brazil. Two of these cases were published as occurring during the week ended August 15 in the PUBLIC HEALTH REPORTS of August 28, 1936, page 1217.

Tunisia—Tunis.—Two cases of plague, 1 case on August 21, and 1 case on August 26, 1936, have been reported in Tunis, Tunisia.

United States—Utah.—A report of plague-infection in Utah appears on page 1279 of this issue of PUBLIC HEALTH REPORTS.

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