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EFFECTIVE QUARANTINE SURVEILLANCE

By JOHN J. SIPPY, M. D., *District Health Officer, Stockton, California*

Surveillance, as a substitute for detention, is at present practiced in maintaining quarantine control of aircraft passengers from foreign ports. Since, to be effective, this procedure must involve the close and ready cooperation of all parties concerned, it is believed by the writer that the publication of the following report of a specific case will be of value in demonstrating the practicability of such cooperation.

A citizen of California had been employed for 6 months, prior to leaving South America for the United States, on a road-building project between San Lorenzo and Mototan, Venezuela. During that period he suffered two attacks of malarial fever and one of dysentery. Following enlargement of the inguinal glands, accompanied by slight fever, beginning about January 24, he was hospitalized at Maracaibo from January 31 to February 11. The medical officer in charge reported an absence of history of primary lesion. Kahn reaction on February 2 was negative, as was also the reaction to Frei antigen. The patient, however, received 9 injections of an organic antimony compound on the presumptive diagnosis of "tropical bubo."

On the morning of February 11 the patient left Maracaibo by airplane, arriving at Miami Beach, Fla., 11 hours later. His temperature on arrival was normal, and so he was not detained by the quarantine officer. During the night he suffered a chill, followed by unrecorded fever. Despite this illness, he left by plane from Miami Beach at noon on February 12. The travel schedule called for his arrival at Oakland on February 14, at noon; but owing to storm conditions, arrival was delayed 22 hours. While en route he suffered repeated chills and high temperatures.

Under date of February 12, this office was apprised by telegram from the U. S. Public Health Service at Miami Beach, Fla. that this passenger had passed through Miami en route to Stockton; and, since he came from a suspected yellow fever area, it was requested that he be kept under surveillance until February 18, and that development of any fever be reported. Contact with his family was established on February 13.

The patient arrived at Oakland, Calif., by plane at 10:15 a. m. on February 15, via Chattanooga, Indianapolis, Chicago, and Salt Lake City, and reached Stockton, Calif., by auto at noon. This office was advised of his arrival at 1:30 p. m. A slight delay was due to the fact that, while en route from Oakland to Stockton, the patient had a severe chill with fever, nausea, and vomiting, and was made comfortable before his wife telephoned.

When the patient was visited at 2 p. m., he complained of severe lumbar pain, his temperature by mouth was 103.8° F., pulse 130. He had enlarged and tender inguinal glands, but other clinical findings were essentially negative, and there was nothing to suggest the textbook picture of yellow fever. Since blood smears revealed numerous malaria parasites, a diagnosis of malarial fever seemed most logical, and the patient was placed under medical treatment with atabrine and quinine.

Except for one malarial paroxysm on the morning of February 17, the patient has responded to treatment, and his temperature has remained normal since February 18. The inguinal glands have entirely receded.

Laboratory tests for plague, relapsing fever, typhoid fever, and tularaemia were all negative. Examination of a blood smear revealed many malarial parasites (probably aestivo-autumnal), with numerous instances of multiple infection of red cells. The only laboratory finding at variance with the malaria picture was a moderate leucocytosis of 13,000.

The unusual features surrounding this case were as follows:

A. The possibilities of the presence of any one of several communicable infections, namely, yellow fever, plague, malignant malarial (blackwater) fever, and "tropical bubo."

B. The fact that, owing to rapidity of present airplane travel, persons from endemic areas throughout the world may bring such infections to any United States community before characteristic symptoms appear or can be recognized.

C. The vigilance of the United States Public Health Service at ports of entry which provides for prompt notification of local health authorities at the destination of travelers and thus permits the immediate institution of measures for prevention of spread of possible infections.

D. In this instance it was unnecessary to notify health officers at stops that had been made by the patient en route since, in the cold weather prevailing, danger of transmission at those points of any of the quarantinable diseases that exist in South America was negligible. During the summer, however, such notification would have been in order.

STUDIES ON THE TOXINS AND ANTITOXINS OF *CLOSTRIDIUM PERFRINGENS*[1]

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

Introduction

Titrations of *Clostridium perfringens* toxins and antitoxins have yielded very inconsistent results in the hands of various workers. Such results are apparently dependent upon the interrelationship of the various antigenic components of the toxins that have been described. This suggests that the differences observed in the protective action of antitoxins may be due to the absence of certain antigenic components in the toxin used for immunization. A study has therefore been undertaken to determine some of the factors which influence the production of toxin with all the antigenic components for a given type, the nature of these antigens, and their significance in the production of an antitoxin which will give complete protection.

From theoretical consideration there were several possible factors that might be involved. (A) The nature of the toxin produced by a given strain might vary depending on: (1) Period of cultivation, (2) composition of the medium, (*a*) cysteine content, (*b*) meat and meat products in the medium, (*c*) glucose content. (B) If toxins vary in their composition depending on the medium used for cultivating the organisms the homologous antitoxins produced by immunizing with these toxins may vary depending on the presence or absence of the different components. (C) The nature of the different antigenic components may be such that the toxins are unstable.

Several different reactions are used for determining the titers of *perfringens* antitoxins. In 1920 Bengtson (*1*) promulgated a standard for the antitoxin of *Clostridium perfringens* of human origin. The unit was designated as that amount of antitoxin which would neutralize approximately 1,000 minimum lethal doses (m. l. d.) of a *perfringens* toxin. The tests were carried out by inoculating pigeons intramuscularly with different toxin-antitoxin mixtures. In 1930 the official unit for measuring the potency of the antitoxins was changed to one one-hundredth the former standard, as the unitage of antitoxins by the former method fell below 5 per cubic centimeter. This unit was adopted as the international standard (*19*).

Other methods which are used for measuring the titers of *perfringens* antitoxin are: (1) *In vitro* hemolytic titrations (Henry (*21*), Mason and Glenny (*25*)); (2) intradermal necrotic tests (Glenny et al. (*16*)); and (3) the mouse protection test (*10*).

[1] From the Division of Biologics Control, National Institute of Health, and Department of Bacteriology and Parasitology, University of Chicago.

Using the above methods variations have been observed in the measurement of the protective power of certain antitoxins when tested against different lots of toxins. Marked irregularities have been reported with the method of *in vitro* hemolytic titrations (Henry (*21*), Weinberg and Guillaumie (*49*), Glenny (*17*), Prigge (*33*, *34*)). Unsatisfactory results have also been reported with the mouse protection test (Weinberg and Guillaumie (*49*)).

At the National Institute of Health, up to 1934, the routine testing of *perfringens* antitoxins had been carried out by the intramuscular injection of pigeons. This method gave quite consistent results. In 1934 comparative tests of the mouse intravenous method and the pigeon intramuscular method were made (*2*). The results showed a very close correlation of these two methods with the particular toxins and antitoxins used. A short time later when the mouse intravenous method was applied to the routine testing of antitoxins very irregular results were obtained. Great variations in the potencies of certain antitoxins were observed depending on the toxins used. Similar variations were found when the American standard antitoxin was tested against different lots of toxins. Because of these irregularities a study was undertaken to determine the causes for such discrepancies.

Literature

Since *Clostridium perfringens* (*Bacillus aerogenes capsulatus, Clostridium welchii*) was first isolated by Welch and Nuttall in 1892 (*50*) the pathogenic action of the organism has been attributed to a variety of different products. At first the acid, principally butyric, produced in a culture was considered as the cause of the lesions of gas gangrene (*8*). The gas produced in the tissues was also believed to be of importance in the spread of the infection (*27*). In 1917 Bull and Pritchett (*6*) first demonstrated that *Cl. perfringens* produces an exo-toxin when grown under suitable conditions, and they showed that a specific antitoxin could be produced by immunizing animals to the toxin. The toxin has been shown to be actively hemolytic, the hemolysin and the toxin being considered as identical by Ouranoff (*31*), Ford and Williams (*14*), Wuth (*55*), and Mason and Glenny (*25*). Others, however, have observed a multiplicity of the toxin components. In 1920 Weinberg and Nasta (*45*) found that the proportion of hemolysin in toxins of different strains varied considerably. More recently (1930) Schnayerson and Samuels (*35*) in studying the blood changes produced by the hemotoxin of *Cl. perfringens* in pigeons described two hemotoxins which differed in the rapidity of their action. Henry (*21*), in 1923, suggested that the organism in addition to the hemotoxin also produced a myotoxin. He was able to demonstrate a partial dissociation of the two lethal components by adsorption with ground fresh muscle. The muscle was found to reduce the

toxicity though causing little change in the hemolytic activity. As the result of pathological studies, others have described nonhemolytic toxin components and designated them according to their specific action on certain tissues. Weinberg and Barotte (*46*) have described a neurotoxin which caused a degeneration of nervous tissue. Weinberg and Combiesco (*47*) have described a toxic factor which was shown to be specific for the walls of blood vessels.

Still another toxic element attributed to this organism is the "nonspecific toxin" described by Wassermann (*42*), Kojima (*24*), Kendall and Schmitt (*23*), and Walbum and Reymann (*40*). They found that this "toxin" was produced when *Cl. perfringens* was grown in a medium containing glucose. It was not neutralized by the antitoxin and was found to kill mice instantaneously when inoculated intravenously. Kendall and Schmitt (*23*) found this "nonspecific toxin" to be a histamine-like substance.

Prigge (*33*) has found that certain *perfringens* toxins contain two components which correspond to those described by Henry (*21*). He has been able to separate partially these two fractions by precipitating with sodium sulfate and ammonium sulfate (*34*). Prigge has proposed the term "zeta toxin" for the nonhemolytic factor and "alpha toxin" for the hemolytic factor. Previously the term "alpha" had been proposed by Glenny et al. (*16*) for the toxin produced by the *Cl. perfringens* strains of human origin. They believed that the toxin was a single antigenic entity which was hemolytic for red blood cells, lethal for mice, and necrotic following intradermal injections into guinea pigs or rabbits.

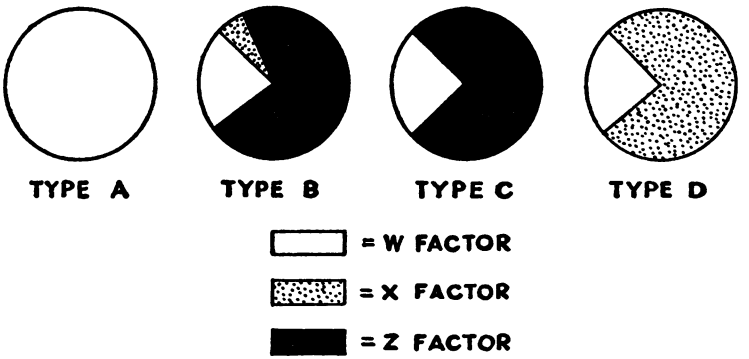
Until 1923 (*15*) only the toxins from strains isolated from human sources had been studied. With the investigation of strains isolated from animal sources the complexity of the toxic components became even more apparent. In 1928 Dalling (*9*) isolated the lamb dysentery bacillus which was found to be similar culturally and morphologically to Welch's organism. He showed, however, that his organism produced a highly potent toxin which was not neutralized by the antitoxin of the classical *perfringens* strains but that its antitoxin would neutralize the toxin of the human strains as well as its own toxin. About one year later, 1929–30, McEwen (*26*) isolated another *perfringens*-like organism which he named *Bacillus paludis*. This bacillus was isolated from sheep suffering from a disease of the enterotoxemia type called "struck." McEwen found that the toxin of his organism was not neutralized by the antitoxin of the *Cl. perfringens* strains of human origin nor would its antitoxin neutralize the toxins of these strains. Further investigations on the anaerobes concerned in diseases of sheep resulted in the isolation of a fourth *perfringens*-like organism. Bennetts (*3*), in 1932, isolated an organism from sheep dying of an enterotoxemia in West Australia. This organism differed

from the other known *perfringens* forms in its toxin, the toxin being neutralized only by its own specific antitoxin. Bennetts named this organism *Bacillus ovitoxicus*.

In 1931 Wilsdon (*51*) made a comparative study of the *perfringens*-like organisms and found that they could be classified according to their toxin-antitoxin reactions. He grouped them all as *Cl. perfringens* (*welchii*). Those of human origin, or the classical *Clostridium perfringens*, were designated as type A; the lamb dysentery organism, or *Bacillus agni*, as type B; *Bacillus paludis* strains as type C; and an organism isolated by himself from an enterotoxemia of sheep as type

FIGURE I

A SCHEMATIC REPRESENTATION OF THE ANTIGENIC COMPONENTS OF THE TOXINS OF THE FOUR TYPES OF CLOSTRIDIUM PERFRINGENS. (WILSDON)



D. Later he showed that his type D and Bennetts' *Bacillus ovitoxicus* were the same (*52*).

A schematic representation of the antigenic components of the toxins of the four types, as given by Wilsdon, is shown in figure 1.

The letter W represents the antigenic factor present in type A toxin. As this factor is present in the toxins of the other three types, the antitoxins of types B, C, and D are capable of neutralizing type A toxin, while type A antitoxin neutralizes only its own toxin. The type B toxin contains, in addition to the W factor, two other factors, Z and X, the Z factor being shared by type C toxin and the X factor by type D toxin. Because of the complexity of the B toxin its antitoxin is capable of neutralizing the toxins of all four types. The type C toxin containing only the two antigenic components Z and W is capable of inciting the production of an antitoxin which neutralizes only type A and type C toxins. Since type D toxin also contains the W factor

in addition to the X factor, its antitoxin is also capable of neutralizing type A toxin as well as its own. Wilsdon's work has been confirmed and extended by others (Glenny et al. (*16*), Borthwick (*4*), Weinberg and Guillaumie (*48*), and Duffett (*13*)).

Experimental

Cultures used.—The cultures used were as follows:

Type A strains:

PB6H, isolated by Bull and Pritchett.
SR12, received from Muriel Robertson.
1633, isolated by Torrey.

Type B strains:

No. 34, Wilsdon's strain.
The *Bacillus agni* of lamb dysentery, isolated from the intestinal content of a lamb (Hall collection).

Type C strains:

No. 3628, Wilsdon's No. 51.
The *Bacillus paludis* of McEwen (No. 40) (American Type Culture Collection).
No. 108A, as above (Hall collection).

Type D strains:

DR_2, Bennetts' strain R_2 of his *Bacillus ovitoxicus* (received from A. T. Glenny).

VARIATIONS OBSERVED IN THE NEUTRALIZING PROPERTIES OF THE AMERICAN STANDARD ANTITOXIN AS DETERMINED BY THE MOUSE PROTECTION TEST

By culturing under identical conditions, several lots of toxin were produced from three different strains of *Cl. perfringens* of human origin, PB6H, SR12, and 1633. These cultures were grown in glucose peptone beef infusion broth. Just before inoculation, the broth was heated in streaming steam for 1 hour to expel the air present, cooled rapidly, and then 0.25 percent glucose was added from a sterile 50 percent solution. Two-liter flasks of the broth were then inoculated with 6 cc. of the supernatant of a 24-hour meat culture. The broth cultures were then layered with sterile vaseline. These were incubated at 37° C. for 10 to 12 hours and then passed through Berkefeld N filters. The crude toxin filtrates were used in some of the tests; in others the toxins were precipitated with 70 percent ammonium sulfate and dried over phosphorus pentoxide. All the dried toxins used in this study were precipitated and dried in a similar manner.

The protection tests were carried out by the intravenous injection of mixtures of toxin and antitoxin into mice weighing 17 to 20 grams.

In order to determine the number of m. l. d. of each toxin neutralized, varying quantities of the toxin were added to a constant amount of antitoxin (0.2 unit). The mixtures were allowed to stand 1 hour at room temperature before being injected into animals.

A protocol of a representative test from one of a series is given in table 1.

TABLE 1.—*The neutralization of 3 different lots of type A Clostridium perfringens toxin by the American standard antitoxin*

Toxin	Amount of toxin	Amount of antitoxin	Number of mice injected	Deaths	Time of death
	M. l. d.	*Unit*			
SR12 filtrate, 0.05 cc.=1 m. l. d..	4	0.2	4	3	10 minutes to 1 hour.
	7	.2	4	4	Immediately.
	10	.2	4	4	Do.
	13	.2	4	4	Do.
	16	.2	4	4	Do.
1633 filtrate, lot I, 0.05 cc.= 1 m. l. d.	4	.2	4	1	15 minutes.
	7	.2	4	3	15 minutes to 11 hours.
	10	.2	4	4	Immediately.
	13	.2	4	4	Do.
	16	.2	4	4	Do.
1633, lot II pptd. and dried, 0.2 mg.=1 m. l. d.	8	.2	6	0	
	11	.2	6	0	
	17	.2	6	0	
	14	.2	6	0	
	20	.2	6	2	3 hours.
PB6H pptd. and dried, 0.6 mg = 1 m. l. d.	2.5	.2	6	1	1 hour.
	5	.2	6	1	Do.
	7.5	.2	6	4	30 minutes to 1 hour.
	10	.2	6	6	5 to 15 minutes.
	12.5	.2	6	5	Immediately.

A. FACTORS WHICH MIGHT INFLUENCE THE NATURE OF THE TOXIN PRODUCED BY A GIVEN STRAIN OF *Cl. perfringens*, TYPE A

1. PERIOD OF INCUBATION

Tests were carried out to determine the effect of the period of incubation as a factor governing the production of a toxin component which is not neutralized by the antitoxin. Three 2-liter flasks of peptone beef infusion broth containing 0.25 percent glucose were heated and cooled as above and then each inoculated with 6 cc. of a 24-hour meat culture of strain SR12. These were incubated at 37° C. for 8, 11.5, and 20 hours. The cultures were then filtered and the toxins precipitated and dried as above. The m. l. d. of each toxin was determined in mice and subsequently tested against the standard antitoxin.

The three toxins were found to be neutralized equally well and with no irregularities. As many as 20 m. l. d. of each toxin were neutralized by 0.2 unit of the antitoxin. This is shown in table 2.

The differences observed in the neutralization of the toxin in this test and in the test shown in table 1 could not be explained on the basis of the data in this preliminary experiment. However, a partial explanation is presented in later experiments dealing with the

hemolysins. It may also be that certain of the toxin components vary in their stability and that precipitating with ammonium sulfate may alter the toxins.

TABLE 2.—*Period of incubation as a factor in the production of a toxin component not neutralized by the standard antitoxin*

SR12 precipitated toxin			Standard antitoxin	Number of mice inoculated	Deaths
Period of incubation	Percent glucose in medium	M. l. d. of toxin			
			Unit		
8 hours	0.25	8	0.2	3	0
		11	.2	3	0
		17	.2	3	0
		20	.2	3	1
11.5 hours	0.25	8	.2	3	0
		11	.2	3	0
		17	.2	3	0
		20	.2	3	2
20 hours	0.25	8	.2	3	0
		11	.2	3	0
		17	.2	3	0
		20	.2	3	2

2. COMPOSITION OF THE MEDIUM

(*a*) *Cysteine content.*—Orr and Reed (*30*) have shown that the addition of 0.1 percent or more of cysteine to chopped meat medium markedly inhibited the production of hemotoxin by *Cl. perfringens* and that hydrogen sulfide has a similar effect. They showed that the effect was related to the metabolism of the organism and not to a direct reaction with the formed toxin. The addition of these concentrations of cysteine was shown to produce a marked drop in the oxidation-reduction potential of cultures during the most active growth period. There were no observable differences in the rate of growth or of the maximum growth obtained. The presence of hemotoxin was determined by titrating against washed sheep red blood corpuscles. No mention is made of the toxicity of the cultures.

Since peptones of different makes differ in their sulfhydryl content as determined by the sodium nitroprusside test, tests were carried out to determine the effect of different peptones, with and without added cysteine, on the hemolysins produced. Four different peptones were tested. These were used in amounts of one percent in beef infusion broth. Cysteine hydrochloride was added in amounts giving 0.015 percent and 0.15 percent concentrations. The media were tubed and sterilized by heating at 15 pounds pressure for 20 minutes. *Cl. perfringens* types A, B, C, and D were grown in the above media for 24 hours at 37° C. and the cultures were then centrifugalized. The supernatant fluid of each was then tested for hemolysins.

Hemolysin titrations of toxins produced in the different peptone media.—All titrations for hemolysins in this study were carried out identically. Five percent suspensions of washed sheep, rabbit, mouse, and human red blood cells were used in 0.5-cc. amounts. Varying quantities of toxin were added to the cells and these were then made up to a volume of 1 cc. with physiological saline. The toxin and red blood corpuscles were incubated for 1 hour at 37° C. and then read for the presence of hemolysis. The least amount of toxin producing complete hemolysis was designated as the minimum hemolyzing dose (m. h. d.).

The results on the hemolysin titrations of the type A toxins produced in the different peptone media are given in table 3.

The results on the hemolysins for the type C toxins were very similar to those of the type A toxins. Types B and D were only slightly hemolytic when grown in the media which gave good hemolysins with types A and C.

TABLE 3.—*The effect of the peptone and of the peptone plus cysteine on hemolysin production by Clostridium perfringens, types A and C*

Peptone	Amount of filtrate	Sheep r. b. c.		Rabbit r. b. c.		Mouse r. b. c.		Human r. b. c.	
		Type A	Type C	Type A	Type C	Type A	Type C	Type A	Type C
	Cc.								
Bacto	0.5	++++	++++	++++	++++	++++	++++	++++	++++
	0.2	++++	++++	++++	++++	++++	++++	++++	++++
	0.1	++++	++++	++++	++++	++++	++++	++++	++++
	0.05	+++	+++	+++	+++	+++	+++	+++	+++
Bacto+0.015 percent cysteine.	0.5	++++	++++	++++	++++	++++	++++	++++	++++
	0.2	++++	++++	++++	++++	++++	++++	++++	++++
	0.1	++++	++++	++++	+++	++++	++++	++++	++++
	0.05	++	++	+++	+++	+++	+++	+++	+++
Bacto+0.15 percent cysteine.	0.5	++++	++++	++++	++++	+++	+++	+++	+++
	0.2	+++	+++	++++	++++	+++	+++	+++	+++
	0.1	+++	+++	+++	+++	++	++	++	++
	0.05	++	++	+++	++	+	+	+	+
Parke-Davis	0.5	++++	++++	++++	++++	++++	++++	++++	++++
	0.2	+++	+++	+++	+++	+++	+++	+++	++
	0.1	++	++	++	++	++	++	++	++
	0.05	+	+	+	+	++	++	+	+
Parke-Davis+0.015 percent cysteine.	0.5	+++	++	+++	+++	+++	+++	+++	+++
	0.2	++	++	++	++	++	++	++	++
	0.1	++	+	++	++	++	++	++	++
	0.05	+	+	+	+	+	+	+	+
Parke-Davis+0.15 percent cysteine.	0.5	+++	++	+++	+++	+++	+++	+++	+++
	0.2	++	++	++	++	++	++	++	++
	0.1	+	+	+	+	+	+	+	+
	0.05	+	+	+	+	+	+	+	+
Proteose	0.5	+++	+++	+++	+++	+++	+++	+++	+++
	0.2	++	++	++	++	++	++	++	++
	0.1	++	+	++	++	++	+	++	++
	0.05	+	+	+	+	+	+	+	+
Proteose+0.015 percent cysteine.	0.5	++	++	++	++	++	++	++	++
	0.2	+	+	+	+	+	+	+	+
	0.1	−	−	−	−	−	−	−	−
	0.05	−	−	−	−	−	−	−	−
Proteose+0.15 percent cysteine.	0.5	+	+	+	+	+	+	+	+
	0.2	+	+	−	−	−	−	−	−
	0.1	−	−	−	−	−	−	−	−
	0.05	−	−	−	−	−	−	−	−

TABLE 3.—*The effect of the peptone and of the peptone plus cysteine on hemolysin production by Clostridium perfringens, types A and C*—Continued

Peptone	Amount of filtrate	Sheep r. b. c.		Rabbit r. b. c.		Mouse r. b. c.		Human r. b. c.	
		Type A	Type C	Type A	Type C	Type A	Type C	Type A	Type C
	Cc.								
Witte	0.5	++++	++++	++++	++++	++++	++++	++++	++++
	0.2	++++	++++	++++	++++	++++	++++	++++	++++
	0.1	++++	++++	++++	++++	++++	++++	++++	++++
	0.05	++++	++++	++++	++++	++++	++++	++++	++++
Witte+0.015 percent cysteine.	0.5	++++	++++	++++	++++	++++	++++	++++	++++
	0.2	++++	++++	++++	++++	++++	++++	++++	++++
	0.1	+++	++	+++	+++	+++	+++	+++	+++
	0.05	++	+	++	++	++	++	++	++
Witte+0.15 percent cysteine.	0.5	++++	+++	++++	++++	++++	+++	+++	+++
	0.2	+++	+++	+++	+++	+++	+++	+++	+++
	0.1	+	+	++	+	++	+	++	++
	0.05	−	−	−	+	+	−	+	+

No marked differences in the hemolytic action of the toxins for the different types of red blood cells were observed. However, differences in the amount of hemolysin formed in the different media were noted. Cultures in the proteose peptone medium gave the poorest hemolysins; proteose peptone plus cysteine hydrochloride almost completely inhibited its production. Berkefeld filtrates of type A proteose cultures containing no hemolysin were tested for toxicity and found to be lethal for mice, indicating the presence of a nonhemolytic toxin component. The toxicity was comparatively low, the m. l. d. for a mouse being from 0.1 cc. to 0.5 cc. when inoculated intravenously.

(*b*) *Meat in the medium.*—The toxin of *Cl. perfringens* has been shown to be readily destroyed in an acid medium. Walbum and Reymann (*40*) have found that a potent toxin could be produced by culturing the organism in peptone veal broth if calcium carbonate were added to keep the pH from becoming too low. Meat has also been used in the medium because of its buffer effect (*2*).

In this study the meats that were used were ground beef heart, ground beef, and ground veal which had been extracted with water for the preparation of infusion broth. In order to use equivalent amounts of each, they were first thoroughly dried under a current of hot air. Each meat preparation was then tested for its buffer effect in the medium and for toxin production. The media were prepared by adding 10 percent of the dried meat to a peptone (any of the well-known brands) beef infusion broth and then adjusting the reaction so the pH after sterilizing would be about 7.8. The medium was sterilized by heating at 20 pounds pressure for 30 minutes.

Bacto beef, a commercial dried powdered meat preparation, was also used in broth. This was used in amounts of 2 to 2.5 percent. The media were prepared as above.

To study the toxin production, flasks of the above media were heated in streaming steam to expel the air and then cooled rapidly and 0.25 percent glucose from a sterile 50 percent solution was added. These were inoculated with a 24-hour culture of the strain under investigation. The cultures were incubated 16 to 20 hours at 37° C. After cultivation the pH was recorded, the cultures centrifugalized and filtered through Berkefeld N candles. The m. l. d. and hemolytic titration were then determined as above.

With all the above media very good toxins were produced with the strains used from the four types of *Cl. perfringens*. However, with type D culture 48 to 72 hours' incubation was required. The pH was in no instance below 6.0–6.2 after the period of cultivation. Only the specific results with the type A strains will be given as this is the chief toxin with which we are here concerned. It may be mentioned, however, that with the other three types very similar results were obtained.

Dried beef heart, beef, and veal in the medium all gave the same results so these will be grouped together as ground meat. This medium yielded toxins with an m. l. d. of 0.0125–0.025 cc. for a 17 to 20 gram mouse when inoculated intravenously. Such toxins were not markedly hemolytic. When tested against sheep red blood cells they were practically nonhemolytic if the readings were made after 1 hour incubation at 37° C. (0.5 cc. of the toxin filtrate giving about a 1 plus hemolysis). If the titrations were read after standing 24 hours at room temperature or in the refrigerator slightly more lysis was observed. Rabbit and human red blood cells were slightly more susceptible to the lysin of this toxin. However, when mouse red blood cells were used in the titrations, hemolysis was very pronounced. The hemolytic action of this toxin will be referred to its action on sheep red blood cells.

Toxins produced in the Bacto beef medium were also found to have high potencies. The m. l. d. of such toxin was often below 0.01 cc. for mice injected intravenously. These toxins were found to be highly hemolytic for all four types of red blood cells employed, mouse red blood cells being the least susceptible.

Decided differences in the rapidity of action of the two toxins have been observed. Toxins produced in meat medium and low in hemolysin for sheep red blood cells were found to be considerably slower in killing than toxins high in hemolysin for sheep red blood cells.

Table 4 gives the results of *in vitro* hemolytic titrations and of the lethal action of the two toxins produced by a single type A strain of *Cl. perfringens* when grown in the two media described above.

TABLE 4.—*Titration of two type A toxins produced by the same culture in different media*

Amount of toxin (cc.)	Hemolysin titration				Toxicity for mice	
	Sheep r. b. c.	Rabbit r. b. c.	Human r. b. c.	Mouse r. b. c.	Amount toxin (cc.)	Mice inoculated
	Toxin filtrate from a type A peptone beef infusion broth culture containing 10 percent dried meat					
0.5	+	++++	++++	++++	0.5	Died.
0.2	+	+++	+++	++++	.2	Do.
0.1	−	++	++	++++	.1	Do.
0.05	−	+	±	++++	.05	Do.
0.025	−	±	−	+++±	.025	Do.
0.0125	−	−	−	++	.0125	Do.
0.006	−	−	−	+	.006	Lived.
	Toxin filtrate from a type A Bacto beef peptone beef infusion broth culture					
0.1	++++	++++	++++	++++	0.5	Died.
0.04	++++	++++	++++	++++	.2	Do.
0.02	++++	++++	++++	++++	.1	Do.
0.01	++++	+++	++	+++	.05	Do.
0.005	+++	+	+	++	.025	Do.
0.0025	+	−	−	±	.0125	Do.
0.001				−	.006	Lived.

The marked differences in the toxins produced in the Bacto beef medium and in the ground meat medium were found, as indicated below, to be due to the presence of lipoid substances in the ground meat. Fifty grams of dried ground veal were finely pulverized and one-half was extracted with ether and alcohol, the method for extracting beef heart for the Kahn antigen (*22*) being used. The veal with the lipoids extracted was then dried and made up into medium using 10 percent of the defatted veal in peptone beef infusion broth, and the pH adjusted to 7.8. As a control the ground veal which had not been extracted with ether and alcohol was made up into medium in the same manner as the defatted veal. Types A and C *Cl. perfringens* were grown in both of these media for 16 hours and then filtered and the filtrate tested for hemolysins.

Both the type A and the type C grown in the defatted veal medium produced toxins with strong hemolysins for sheep, rabbit, and mouse red blood cells, while the filtrates of the cultures grown in the veal medium with the veal that had not been defatted were nonhemolytic for sheep red blood cells, only slightly hemolytic for rabbit red blood cells, but definitely hemolytic for mouse red blood cells. This is shown in table 5.

In order to determine whether the lipoid substances inhibited hemolysin production, or merely masked the hemolysin present, or acted in some way to protect the sheep, rabbit, and human red blood cells, the supernatant fluids of centrifuged cultures grown in ground veal medium were thoroughly extracted with ether and tested

for hemolysins. The toxin remained nonhemolytic for sheep red blood cells and only slightly hemolytic for rabbit and human red blood cells. Reduction with sodium sulfite did not activate hemolysis. Hemolytic controls extracted in like manner remained hemolytic.

TABLE 5.—*The effect of lipoids on hemolysin production by Clostridium perfringens types A and C*

Medium	Amount of culture filtrate used, cc.	Sheep r. b. c.	Rabbit r. b. c.	Mouse r. b. c.
	Type A toxin			
10 percent ground lean veal in beef infusion peptone broth	0.5	+	++	++++
	.2	−	+	++++
	.1	−	−	++++
	.05	−	−	+++±
	.025	−	−	++
Same as above, only the ground veal was extracted with ether and alcohol	.1	++++	++++	++++
	.04	++++	++++	+++
	.02	++++	++++	+
	.01	++++	+++	−
	.005	+++	++	−
	Type C toxin			
10 percent ground lean veal in beef infusion peptone broth	0.5	+	++	++++
	.2	−	+	++++
	.1	−	±	+++
	.05	−	−	++
	.025	−	−	+
Same as above, only ground veal was extracted with ether and alcohol	.1	++++	++++	++++
	.04	++++	++++	+++
	.02	+++	++++	+++
	.01	+++	+++	±
	.005	++	++	−

From these results it appears that the lipoids either inhibit the formation of hemolysin for sheep red blood cells or in some way alter it.

(*c*) *Glucose in the medium.*—Several investigators have found that when *Cl. perfringens* is grown in a medium containing glucose a toxic substance which is not neutralized by the antitoxin is formed. Kojima (*24*) found that this "nonspecific toxin" was directly correlated with the percentage of glucose in the medium, being produced when the sugar content is high, the line of division being fairly constant at 0.5 percent glucose. Walbum and Reymann (*40*) found that it was not formed in measurable quantities in media containing 0.75 percent glucose. However, with a content of 2.25 percent glucose it was formed in considerable quantity.

In our work three type A strains, SR12, PB6H, and 1633, were grown in flasks of peptone beef infusion broth containing glucose in the following percentages: 0.25, 0.5, 1.0, 2.0, and 2.25. These were

incubated 12 hours, filtered through Berkefeld N candles, and the toxin filtrates tested for their m. l. d. Neutralization of these toxins was then determined by testing against two different antitoxins, one high in antihemolysin and the other low in antihemolysin as determined by titrations against toxins highly lytic for sheep red blood cells. (This will be shown in the section on the antigenic relationship of the hemolysins.)

As seen in table 6 all the toxins, even those produced in media containing a high percentage of glucose, were neutralized by antitoxin 229. With antitoxin AS_1 no protection was given against the toxins produced in the medium containing 1 or 2 percent glucose; as few as 2.5 m. l. d. were not neutralized. However, when this same antitoxin was tested against the toxins made in the media containing 0.25 and 0.5 percent glucose the mice were protected. Antitoxin AS_1, as contrasted with antitoxin 229, had very little antihemolysin for the lysin for sheep red blood cells.

TABLE 6.—*The neutralization of the "nonspecific" toxin produced by Cl. perfringens (PB6H) when grown in a media containing different percentages of glucose*

Toxin				Antitoxin			Toxicity for mice	Time of death (minutes)	Hemolytic action of toxin-antitoxin mixtures on red blood corpuscles			
Percent glucose	M. l. d. for sheep r.b.c. (cc.)	Amount (cc.)	Number m. l. d.	Number	Amount (cc.)	Dil.			Sheep	Mouse	Rabbit	Human
0.25	0.02	0.05	2.5	229	0.2	1:2	Lived	------	−	−	−	−
		.125	5.0	229	.2	1:2	do	------	−	−	−	−
		.25	10.0	229	.2	1:2	do	------	−	−	−	−
.5	.04	.025	2.5	229	.2	1:2	do	------	−	−	−	−
		.05	5.0	229	.2	1:2	do	------	−	−	−	−
		.1	10.0	229	.2	1:2	do	------	−	−	−	−
1.0	.1	.25	2.5	229	.2	1:2	do	------	−	−	−	−
		.5	5.0	229	.2	1:2	do	------	++	−	±	+
		1.0	10.0	229	.2	1:2	Died	90	+++	+	+++	+++
2.0	.1	.25	2.5	229	.2	1:2	Lived	------	−	−	−	−
		.5	5.0	229	.2	1:2	do	------	−	−	−	−
		1.0	10.0	229	.2	1:2	do	------	++	−	++	++
.25	.02	.05	2.5	AS_1	.2	1:25	do	------	+++	±	+++	+++
		.125	5.0	AS_1	.2	1:25	do	------	++++	±	++++	++++
		.25	10.0	AS_1	.2	1:25	do	------	++++	±	++++	++++
.5	.04	.025	2.5	AS_1	.2	1:25	do	------	+++	−	+++	+++
		.05	5.0	AS_1	.2	1:25	do	------	++++	±	++++	++++
		.1	10.0	AS_1	.2	1:25	do	------	++++	+	++++	++++
1.0	.1	.25	2.5	AS_1	.2	1:25	Died	20	++++	++	++++	++++
		.5	5.0	AS_1	.2	1:25	do	10	++++	+++	++++	++++
		1.0	10.0	AS_1	.2	1:25	do	1	++++	++++	++++	++++
2.0	.1	.25	2.5	AS_1	.2	1:25	do	40	++++	++	++++	++++
		.5	5.0	AS_1	.2	1:25	do	30	++++	+++	++++	++++
		1.0	10.0	AS_1	.2	1:25	do	5	++++	++++	++++	++++

Longer periods of incubation appear to have little effect on the production of a "nonspecific toxin." Cultures grown in meat medium or broth with added calcium carbonate, containing 2.25 percent glucose and incubated for 20, 24, 30, 36, and 41 hours, showed very little toxicity. In some instances 0.5 cc. was found to kill mice instantly when inoculated intravenously but the toxin was neutralized by antitoxin.

B. THE ANTIGENIC RELATIONSHIP OF THE HEMOLYSINS

In order to determine the toxicity and the antigenicity of the two hemolysins prepared in the different media, antitoxins were prepared against the two toxins. Rabbits were immunized against the toxins of types A, B, C, and D *Cl. perfringens*, using strongly hemolytic filtrates from peptone beef infusion broth cultures and Bacto beef cultures and with filtrates from cultures grown in ground meat medium which were nonhemolytic for sheep red blood cells.

Formalinized toxins (0.3 percent formaldehyde added and then incubated for 48 hours at 37° C.) were used for immunizing the rabbits. In order to assure good antihemolytic titers in the sera, when the rabbits had reached a fair degree of immunity, fresh toxin filtrates were used instead of the formalinized toxins. A total of 36 injections was given to each rabbit. Two rabbits were immunized against each toxin as follows:

Rabbit	Strain	Type	Medium
243	PB6H	A	Ground meat medium.
67	PB6H	A	Do.
229	PB6H	A	Peptone beef infusion broth and Bacto beef.
235	PB6H	A	Do.
164	108A	C	Ground meat medium.
244	108A	C	Do.
86	108A	C	Peptone beef infusion broth and Bacto beef.
241	108A	C	Do.
171	34	B	Ground meat medium.
181	34	B	Do.
18	34	B	Peptone beef infusion broth and Bacto beef.
16	34	B	Do.
226	DR_3	D	Ground meat medium.
286	DR_3	D	Do.
11	DR_3	D	Peptone beef infusion broth and Bacto beef.
15	DR_3	D	Do.

1. SPECIFICITY OF ANTIHEMOLYSINS

The antigenic relationship of the two hemolysins was determined by hemolytic titrations and by mouse intravenous inoculations of toxin-antitoxin mixtures. Each rabbit antiserum was tested against the two hemolysins produced by the homologous type. The hemolytic titrations and the protection tests were carried out as in the previous experiments. Two mice were used to each dose of toxin-antitoxin mixture. The size of the inoculum was kept to 1 cc. when possible.

It was found that antitoxins 235 and 229 which were prepared by immunizing with strongly hemolytic type A toxins gave complete protection against both hemotoxins of type A. Antitoxins 67 and 243 prepared by immunizing with the toxins that were not lytic for sheep red blood cells neutralized only their homologous toxins. The results are given in table 7.

TABLE 7.—*The antigenic relationship of the two types of hemolysins as determined by hemolytic titration with sheep red blood cells and by neutralization of the lethal effect*

Antitoxin number, 0.2 cc. of 1:10 dilution	Type A hemolytic toxin			Type A toxin, nonhemolytic for sheep r. b. c.		
	Amount toxin, m. l. d.	Hemolysin for sheep r. b. c.	Protection for mice	Amount toxin, m. l. d.	Hemolysin for sheep r. b. c.	Protection for mice
67	2	++	Lived	2	—	Lived.
	4	++++	Died	4	—	Do.
	8	++++	do	8	—	Do.
	16	++++	do	16	—	Do.
243	2	++++	do	2	—	Do.
	4	+++	do	4	—	Do.
	8	++++	do	8	—	Died.
	16	++++	do	16	—	Do.
235	2	—	Lived	2	—	Lived.
	4	—	do	4	—	Do.
	8	—	do	8	—	Do.
	16	—	do	16	—	Do.
	20	—	do	20	—	Do.
229	2	—	do	2	—	Do.
	4	—	do	4	—	Do.
	8	—	do	8	—	Do.
	16	—	do	16	—	Do.
	20	—	Died	20	—	Died.

The results were similar with types B, C, and D toxin-antitoxin mixtures. With types B, C, and D, as with type A, if the hemolysins produced in the broth cultures were not neutralized by the antitoxin, the toxin-antitoxin mixtures were toxic for mice when inoculated intravenously. The type B and D toxins of the strains used were never highly hemolytic for sheep red blood cells so the differences in the hemolytic and nonhemolytic toxins were not as pronounced as with the types A and C strains. These tests show the importance of producing antitoxins which have antihemolysins for the different types of hemolysins produced by *Cl. perfringens*.

2. HEMOLYSIN ABSORPTION AS FURTHER EVIDENCE OF THE SPECIFICITY

Toxin filtrates from ground meat cultures, broth cultures and Bacto beef cultures were absorbed with stroma from sheep, rabbit, and mouse red blood cells prepared according to the method of Pascucci (*32*). The absorption of the hemolysins was carried out by incubating 40 m. h. d. of the toxins with 50 milligrams of the different dried stroma. (The m. h. d. was determined with sheep red blood cells for the broth and Bacto beef toxins, while mouse red blood cells were used for the toxin produced in the ground meat medium.) The stroma and toxin were incubated overnight at 10° C., then one hour at 37° C. The toxins were then centrifugalized in order to remove the stroma and the clear supernatant tested for lysins present for sheep, rabbit, and mouse red blood cells.

The results of the hemolysin absorption tests of the three type A toxins are given in table 8.

TABLE 8.—*Absorption of the hemolysins of type A toxins with dried pulverized stroma of rabbit, mouse, and sheep red blood cells (40 m. h. d. of toxin + 50 mg. stroma)*

Medium used for producing the toxin	Red blood cell stroma	Hemolytic titrations		
		Sheep red blood cells	Rabbit red blood cells	Mouse red blood cells
Broth	Sheep	−	±	−
	Rabbit	−	±	+
	Mouse	−	±	+
Ground meat	Sheep	−	+±	++++
	Rabbit	−	+±	++++
	Mouse	−	+±	++++
Bacto beef	Sheep	−	+++±	++++
	Rabbit	−	+++±	++++
	Mouse	−	+++±	++++

The hemolysin present in broth culture filtrates was absorbed out by each of the three different types of red blood cell stroma used, the filtrate becoming nonhemolytic for all three types of cells. The absorption was nonspecific; that is, the lysin for sheep red blood cells was absorbed out by rabbit, mouse, and sheep red blood cell stroma. The same was true of the hemolysin for the rabbit and mouse red blood cells.

With the toxins prepared in the Bacto beef medium, which also had hemolysins for all three types of red blood cells, only the hemolysin for the sheep red blood cells was completely absorbed out, this absorption being nonspecific as all three types of red blood cell stroma removed it. The hemolysins for the mouse and the rabbit red blood cells were only partially absorbed.

The hemolysin of the toxin filtrates from cultures in ground meat medium differed from the hemolysin in the broth cultures in that it was not absorbed by red blood cell stroma. Only a very slight absorption was noted in testing for the hemolysin for rabbit red blood cells, while with mouse red blood cells no absorption appears to take place with any red blood cell stroma. The hemolysin was not removed even when the number of m. h. d.'s was decreased to 5 m. h. d. and the red blood cell stroma increased to 100 milligrams.

The same results were obtained with the hemolysins of types B, C, and D toxin filtrates.

C. THE NATURE OF THE TOXIN COMPONENTS OF *Clostridium perfringens* TYPE A

1. THE INACTIVATION OF THE HEMOLYSINS BY FILTRATION

Inactivation of hemolysins as a result of filtration through Seitz filters has been observed by Chopra and Roy (7). They explained the inactivation as due to the surface action of the asbestos which eliminates the active principle by adsorption or by altering its nature

altogether. A similar inactivation of the hemolysins of *Cl. perfringens* has been observed in our work as a result of filtering through collodion or cellophane membranes.

Filtration of the toxin of *Cl. perfringens* through graded membranes will be discussed in another communication.

Membranes through which hemolytic toxins had been filtered and which had completely removed the hemolysins from the filtrate were washed in a small volume of saline, about one-fourth that of the toxin filtered, and the washings tested for hemolysin. Although slight hemolytic activity could be demonstrated it was never of the original titer. Attempts to recover the hemolysins by grinding the mem branes or by eluting, using buffers with a range from pH 5.0 to pH 9.4, were negative.

It was found that complete inactivation of the hemolysins on the membrane could be brought about by filtering a quantity of physiological saline through the membrane after filtering the toxin, thus washing it free of the broth present.

2. ACTIVATION OF THE HEMOLYSINS

The inactive hemolysins on the membrane could be recovered by washing the membrane in a small amount of physiological saline. This, as well as the first filtrate (the broth filtrate), was nonhemolytic. However, it was found that if the two were mixed, the mixture became hemolytic. This suggested the possibility of a cohemolysin in the filtrate. A similar activation of the hemolysins recovered from the membrane was produced by adding broth or 0.05 percent cysteine hydrochloride. The addition of two amino acids without SH groups, glycine and alanine, did not bring about an activation. It may be assumed, therefore, that the activation is due to a reduction of the hemolysins or that the hemolysins are capable of bringing about a lysis of red blood cells only when in a reduced medium. That the activation is due to a reduction either of the hemolysins or of the medium in which suspended can be further shown by bubbling oxygen through an active preparation. This procedure was found completely to inactivate the hemolysins, the reaction being reversible.

The activation of enzymes with reducing substances such as hydrogen cyanide, hydrogen sulfide, or organic substances containing sulfhydryl groups in the reduced form has been extensively studied (*18*, *28*, *39*, *41*, *43*). Hellerman, Perkins, and Clark (*20*) believe that the phenomenon associated with the reversible inactivation of some enzymes is due to a direct action of the sulfhydryl groups of the enzyme itself; the enzyme in its reduced form, for example En-SH, being active while the En-S-S-En oxidized form is inactive.

A reversible inactivation of hemolysins similar to that described for enzymes has also been shown. Neill (*29*) found that pneumococcal

hemolysin which had been inactivated by air or hydrogen peroxide may be reactivated by the use of reducing substances. Shwachman, Hellerman, and Cohen (*37*) showed that the pneumococcal hemolysin is active when reduced and may be reversibly inactivated by operations that are known to convert sulfhydryl compounds to the corresponding dithio derivatives or to mercaptides.

If an enzyme or a hemolysin possesses SH groups which determine its activity the active form of a purified enzyme or hemolysin might be expected to give a positive nitroprusside test and the inactive form to become activated on the addition of hydrogen cyanide.

In testing the effect of hydrogen cyanide on *perfringens* hemolysin it was found that at pH 7.0 concentrations of 0.025, 0.05, and 0.1 percent had no effect on the activation of the hemolysin inactivated by filtration. Concentrations of 0.1 and 0.05 percent were found to be inhibitory to the action of active hemolysin but 0.025 percent had no effect. Hydrogen cyanide has been shown to have an inhibitory effect on certain enzymes (*36*, *38*). Results of the activation of *perfringens* hemolysin are given in table 9.

TABLE 9.—*The action of activating substances on the hemolysin inactivated by filtering through a cellophane membrane*

Hemolysin	Dilution	Amount, cc.	Hemolytic activity		
			Sheep r. b. c.	Rabbit r. b. c.	Mouse r. b. c.
Hemolysin separated from broth by filtration, recovered with saline.	Undiluted	0.5	−	−	−
		.2	−	−	−
		.1	−	−	−
		.05	−	−	−
Above+nonhemolytic filtrate	1:1	.5	++++	++++	++++
		.2	++++	++++	++++
		.1	++++	++++	+++
		.05	++	++	+
Above hemolysin+broth	1:1	.5	++++	++++	++++
		.2	++++	++++	++++
		.1	++++	++++	+++
		.05	++	++	+
Above hemolysin+0.05 percent cysteine hydrochloride.	Undiluted	.5	++++	++++	++++
		.25	++++	++++	++++
		.1	++++	++++	+++
		.05	++	++	+
Above hemolysin+0.05 percent KCN +0.025 percent KCN.	do	.5	−	−	−
		.5	−	−	−
Original active hemolysin+0.05 percent KCN+0.025 percent KCN.	do	.5	−	−	−
		.5	++++	++++	++++

3. A NONHEMOLYTIC TOXIN COMPONENT

At times the filtration of *Cl. perfringens* type A toxins through collodion membranes resulted in the separation of a toxin component in the filtrate which was nonhemolytic for rabbit, mouse, and sheep red blood cells as determined by *in vitro* titrations. This toxin was lethal

for mice, the m. l. d. being from 0.1 cc. to 0.5 cc. It was impossible to obtain consistent results. The production and filtration of the nonhemolytic component is dependent upon factors not yet determined.

Discussion

The variations observed in the neutralizing properties of certain *Cl. perfringens* type A antitoxins when tested against different lots of type A toxins appear to be due to differences in the composition of the toxins. The nature of the toxin produced by a given strain has been shown to be dependent on the medium.

Recently Dalling and Ross (*12*), in studying the factors influencing the production of the various toxins of the *Cl. perfringens* group, have shown the importance of meat in the production of the epsilon factor (Glenny et al.) or Wilsdon's X factor by types D and B cultures. They found that an epsilon toxin of high value could be obtained by culturing in a medium with 50 percent meat by volume and that with type B cultures it was practically negligible in the absence of meat.

Since the medium used in culturing the *Cl. perfringens* group determines to a large extent the toxin components formed, the stability of the types is also more or less influenced by the medium. Dalling (*11*) has reported a type B which became permanently altered so that it lost its capacity to produce the epsilon factor, and so became toxicogenically a type C. Dalling and Ross (*12*) have reported a type C which at times failed to produce any beta toxin (Glenny et al.). It differed from the type A cultures in that it produced a delta hemolysin (Glenny et al.) as well as the alpha toxin. Borthwick (*5*) has described the conversion of a type D to a type A.

A résumé of the toxin components which have been described for the toxins of the *Cl. perfringens* group is as follows:

Type A.—Alpha toxin (Glenny et al.) or the W factor (Wilsdon); this is hemolytic, lethal, and necrotic; and a zeta (Prigge) or nonhemolytic toxin factor.

Type B.—Alpha toxin; beta toxin (Glenny et al.), or the Z factor (Wilsdon); this is lethal and necrotic; a gamma toxin (Glenny et al.) and an epsilon toxin (Glenny et al.), or the X factor (Wilsdon). The gamma and epsilon toxins are also lethal and necrotic.

Type C.—Alpha, beta, gamma, and a delta toxin (Glenny et al.). The delta fraction is a hemolysin and is believed to be nontoxic.

Type D.—Alpha and epsilon factors.

In this study, although many of the details remain to be worked out, our results show that the toxin of *Cl. perfringens* type A when produced under suitable conditions is a complex substance made up of at least three components, two hemotoxins and a nonhemolytic toxin. The production of *Cl. perfringens* type A antitoxins with antibodies for all the toxin components depends on the use of a toxin pro-

duced in a medium which yields all the toxin components. A medium made with 10 percent dried defatted ground meat or 2.0 to 2.5 percent Bacto beef in a peptone beef infusion broth has been found suitable for this purpose.

The factors which influence the production of the nonhemolytic toxin have not been determined. Its presence as determined by filtration through collodion and cellophane membranes was quite irregular. This irregularity of the nonhemolytic toxin in the filtrates may have been due to differences in the physical properties of the membranes; some may have retained it along with the hemolysins while others allowed it to pass; or it may be that the nonhemolytic toxin is formed only after a definite period of growth and is unstable. Nonhemolytic filtrates which were toxic could not be made hemolytic by reducing, using the usual methods of reduction. The two other toxin components, the hemotoxins, may be reversibly inactivated. The lytic activity of the hemolysins appears to be controlled by the state of oxidation of either the lysins themselves or of the medium in which they are suspended. When the hemolysins were completely separated from the broth in which they were suspended they lost all their lytic activity. On the addition of the nonhemolytic filtrate to the inactivated hemolysins the lytic property of the hemolysins returned. Broth and cysteine hydrochloride were found to have the same activating effect. The activation of the hemolysin may have been due to one of the following reactions: (1) The addition of sulfhydryl groups to the hemolysin, (2) a reduction of the sulfhydryl groups in the hemolysin, (3) a reduction of the medium in which the hemolysin was suspended. It was not determined which reaction took place. It was shown, however, that potassium cyanide added to the inactive hemolysin did not activate it or give a positive nitroprusside test showing that the S-S groups were changed to SH groups. It may be added, however, that this test has been reported as not altogether reliable in testing for the thiol group (*37*).

The hemolysin that is lytic for sheep red blood cells seems to be the less stable of the two hemotoxins. Under certain conditions precipitating with ammonium sulfate appears to inactivate the hemolysin to a marked degree.

The "nonspecific" toxin resulting from the cultivation of *Cl. perfringens* type A in media containing from 1 to 2.25 percent glucose was found to be neutralized by antitoxins which had high antihemolytic titers.

Summary

Cl. perfringens type A has been shown to produce at least three different toxin components which are dependent on the type of medium used for culturing the organism. Two of the components are hemotoxins which can be separated by absorption on dried red blood cell

stroma and by filtration. One is lytic for sheep red blood cells while the other has little action on them after 1 hour's incubation at 37° C. These two hemotoxins were shown to be antigenically different. Lipoids were found to inhibit the formation of the hemotoxin which is lytic for sheep red blood cells.

The hemotoxins may be reversibly inactivated by separating them from the broth in which they are suspended or by bubbling oxygen through the active hemolysins. The hemotoxins may be separated from the broth by filtering through collodion membranes. Activation of the hemolysins can be brought about by introducing substances containing SH groups.

A nonhemolytic toxin component was found in some of the type A filtrates. It was possible to separate this toxin from the hemolysins by filtering through graded membranes.

The "nonspecific toxin" resulting from the cultivation of *Cl. perfringens* type A in media containing from 1 to 2.25 percent glucose was found to be neutralized by antitoxins having high antihemolytic titers for the two types of hemolysins.

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THE EXPERIMENTAL PATHOLOGICAL CHANGES PRODUCED BY THE TOXIN OF *CLOSTRIDIUM HISTOLYTICUM* IN ANIMALS[1]

By Joseph G. Pasternack, *Passed Assistant Surgeon*, and Ida A. Bengtson, *Senior Bacteriologist, United States Public Health Service*

A study of methods suitable for the standardization of *Clostridium histolyticum* antitoxin (*1*) and experimental work in the production of a potent *histolyticum* toxin (*2*) afforded us an opportunity to study the pathological changes produced by the toxin in a large number of mice, guinea pigs, rabbits, and rats.

In addition to the marked proteolytic effects possessed by the toxin, Stewart (*2*) has demonstrated a strong hemolysin in 2 percent glucose broth cultures of the micro-organism.

The anaerobe *Cl. histolyticum* was first described by Weinberg and Seguin (*3*) in 1916 in a study of the bacterial flora of war wounds. It is a Gram positive, motile, spore-forming, somewhat pleomorphic

[1] From the Pathologic Service, U. S. Marine Hospital, New Orleans, La., and the Division of Infectious Diseases, National Institute of Health.

organism. The spores are oval, occurring in the subterminal position and swelling the rod. It is less strictly anaerobic than other anaerobic organisms of the gas gangrene group, growing to a slight extent on the surface of plain agar and blood agar. Carbohydrates are not fermented. Milk, coagulated albumen, blood serum, brain and meat media are digested. Tyrosin crystals occur in meat media cultures. The organism is remarkable for its rapid lytic action on living tissue, which is in marked contrast to its slower action on nonliving tissue. This property is exhibited by the bacteria-free filtrate as well as by cultures, though to a lesser extent. The active lytic action on living tissue has suggested the experimental use of filtrates in the treatment of malignant growths, but no conclusive results have thus far been obtained.

The subcutaneous or intramuscular inoculation of cultures or filtrates induces the well-known, rapidly progressing local lesion in guinea pigs as described by Weinberg and Seguin (*3*), Nasta (*4*), and Combiesco (*5*).

The experimental pathological changes produced by the specific toxin of *Clostridium histolyticum* in animals have not been previously reported.

The reports of Nasta (*4*) and Combiesco (*5*) describe the local lesion in guinea pigs following subcutaneous and intramuscular inoculation of whole cultures of the organism.

Their observations agree in regard to the rapid and progressive swelling of the part, the intense edema that dissects and tears apart the tissues, and the purplish discoloration of the skin and hemorrhagic liquefaction of the soft parts. The digestive process may clean the bone of soft tissue, and spontaneous amputation of the limb may result. The animals may die in 12 to 24 hours, or linger for days in fairly good health and die of secondary infection.

Nasta (*4*) traced the evolution of the lesion in the thigh muscle of guinea pigs over a period of hours. He observed that the lytic effects spread far beyond the distribution of the micro-organism itself. Obviously this was due to the permeation of the toxin produced by the organism. He was of the opinion that the toxin had a specific and elective affinity for voluntary muscle tissue.

Beckwith and MacKillop (*6*) first entertained the idea that the toxin may be absorbed into the circulation and thereby produce lytic phenomena in tissues of the body distant from the site of inoculation of the organism. They inoculated whole cultures of the organism into the gluteal muscles of a small group of guinea pigs and after 48 hours autopsied them. They make no mention of gross findings. Histologic examination of the kidneys, liver, spleen, lungs, adrenal glands, and heart disclosed foci of liquefaction necrosis, exudation of fibrin, and hemorrhage in some of the animals.

The present report is based on the gross observation and microscopic study of 135 mice, 56 rats, 78 guinea pigs, and 40 rabbits. Many more animals were actually utilized, but they were discarded because they died during the night or showed intercurrent infection.

About 75 percent of the animals were inoculated intravenously with toxin alone or with various mixtures of toxin and antitoxin. The remainder received variable doses of potent toxin subcutaneously or intramuscularly.

The toxin used for inoculating the animals was prepared by incubating cultures in meat infusion broth made with Witte's peptone of a reaction of pH 7.4 to 7.6 for 13½ hours, filtering, and precipitating with ammonium sulfate.

The variation in length of survival of animals receiving approximately the same doses of toxin afforded an opportunity to observe the effects of the toxin after different time intervals.

All tissues were fixed in Orth's fluid. Sections were stained with Weigert's acid iron chloride hematoxylin and Van Gieson's picrofuchsin, and with a buffered Romanowsky stain (7). The latter stain is especially valuable for studying early and slight retrograde degenerative changes. Fibrin was stained according to Weigert. Herxheimer's rapid (Sudan IV) method was used to demonstrate fatty changes in the viscera.

PATHOLOGICAL CHANGES IN GUINEA PIGS, RABBITS, RATS, AND MICE AFTER INTRAVENOUS INOCULATION

Gross examination.—Only occasional animals showed gross lesions.

In 5 guinea pigs that died after 28, 45, 46, 80, and 117 hours, the following lesions were present, respectively: Petechial hemorrhages in both kidneys; hemorrhagic infarction of part of the spleen; bilateral hemorrhagic necrosis of the adrenal glands; intense pulmonary edema; and miliary focal necroses of the liver.

Three rabbits that died after 24, 30, and 51 hours showed, respectively, petechial hemorrhages of the kidneys, hemorrhagic pneumonia of the left lung, and foci of hepatic necrosis.

There were no demonstrable gross lesions in the mice and rats.

Microscopic examination.—Microscopic lesions in one or more organs were found in 56 percent of the mice, 33 percent of the rats, 69 percent of the guinea pigs, and 65 percent of the rabbits.

Spleen: This organ showed degenerative changes more regularly than any other. All of the mice that survived 12 hours or longer, about one-half of the guinea pigs that survived over 36 hours, about one-third of the rabbits that lived over 36 hours, and a few of the rats showed some type of lesion.

Congestion of the pulp was frequently present and frank hemorrhages that obscured the architecture were occasionally seen. Occasionally the sinuses were intensely distended with serous or serosanguinous fluid. In 5 mice and 3 guinea pigs foci of pulp thrombosis were seen, and in 1 guinea pig there was massive hemorrhagic infarction.

Hemolysis and degenerative changes in the red blood cells were seen in animals that survived 3 to 4 days. Considerable quantities of free and phagocytosed hemosiderin were frequently present.

Pyknosis and karyorrhexis of the lymphocytes in the Malpighian corpuscles and pulp was the most frequent lesion. This degenerative change was present in various degrees. Variable numbers of follicles showed pyknosis and fragmentation of their lymphoid cells. Not infrequently several or many follicles were converted into collections of nuclear debris, among which large, pale reticulum cells filled with nuclear fragments were present.

The pulp lymphocytes showed degenerative changes less often. Occasionally the pulp was edematous, considerably depleted of lymphocytes and contained macrophages filled with nuclear debris. These clear areas might be interpreted as foci of liquefaction, but the stroma was intact and there was no evidence of tissue necrosis other than degenerative changes in the lymphocytes.

In all of the animals, but more particularly in rabbits, collections of polymorphonuclear leucocytes were demonstrable when advanced degenerative changes were present.

The progress of the intoxication had no notable effect on the number of megakaryocytes present. They fluctuated considerably in various stages in all animals. Frequently they showed nuclear pyknosis and hyalin or hydropic degeneration of the cytoplasm.

Fibrin was demonstrated in occasional areas of pulp thrombosis and hemorrhage.

Rather frequently the central arterioles showed complete hyalinization of their wall. A similar change was also present in the arteries of several guinea pigs. The endothelial lining was absent in these blood vessels.

Kidneys: Lesions of some type were present in all of the animals that received the larger doses of toxin and survived over 24 hours. Roughly, the degree and extent of the renal damage was proportional to the survival time.

The degenerative changes affected principally the convoluted tubules and ascending limbs of Henle's tubules. The epithelium of these tubules showed all grades of swelling, granular and hydropic degeneration. Albuminous coagulum was frequently present in the lumen.

Some degree of colloid droplet degeneration was present in the majority of the animals. This varied from minute eosinophilic hyaline droplets more or less intermingled with the cytoplasmic granules, to large coarse colloid droplets that ruptured the epithelium and escaped into the lumen of the tubules. Fusion of these droplets resulted in the hyalin casts that were seen in many tubules. Rarely minute fat droplets were demonstrated in the epithelium.

Necrosis of tubular epithelium was present in an occasional animal. This lesion varied from increased cytoplasmic oxyphilia and pyknosis of isolated epithelial cells or groups of cells, to massive coagulation necrosis of segments of tubules. Extensive necrosis was rarely seen.

Specimens that showed considerable tubular degeneration occasionally showed scattered glomerular lesions. Desquamation of the capsular epithelium, serous coagulum in the capsular space, and rarely collections of erythrocytes were present. Hyalin thrombi were found in segments of occasional loops and rarely partial thrombonecrosis of a glomerular loop was encountered.

Rather frequently there was marked congestion of the blood vessels, moderate interstitial edema, focal hemorrhages deep in the cortex, with only minor degenerative changes in occasional tubules.

The capillaries and larger blood vessels occasionally showed deposits of hemosiderin in their wall but no lesions.

Heart: Lesions were present in 19 mice, 5 guinea pigs, and 4 rabbits.

Irregular interstitial edema of moderate severity and scattered small focal interstitial hemorrhages were present. Segments of muscle fibers and groups of fibers showed swelling, hydropic or coarse granular degeneration, and more or less deformity. Necrobiotic lesions were not found. Fat droplets were not demonstrable within muscle fibers.

Liver: Animals that died in less than 50 hours rarely showed lesions. The Kupffer cells were frequently filled with hemosiderin. Occasionally marked diffuse congestion was present. Scattered foci of necrosis of hepatic cells were found in 10 percent of the animals. These consisted of small scattered foci of coagulation necrosis. The hepatic cells in these areas showed various degrees of cytoplasmic oxyphilia and nuclear pyknosis, or hyalin eosinophilic coagulation and karyolysis. Occasionally hyalin or fibrin thrombi were demonstrable in these areas. The larger foci of necrosis were frequently associated with hemorrhage and hematogenous pigmentation of the bordering hepatic cells.

In the rabbits the more advanced foci of necrosis were infiltrated by polymorphonuclear leucocytes.

Lymph nodes: Lymph nodes from various regions of a small number of animals were examined. The tracheobronchial and mesenteric nodes showed moderate congestion and edema. Occasionally small

foci of pyknosis and karyorrhexis of lymphocytes were seen. Macrophages filled with nuclear debris were present in the sinuses.

Adrenal glands: Mice and rats rarely showed significant pathologic changes. About one-third of the guinea pigs and about 10 percent of the rabbits showed some type of lesion.

Moderate interstitial edema, congestion, and scattered hemorrhages in the cortex were present. In a few of the animals large disrupting hemorrhages were seen at the junction of cortex and medulla, and occasionally in the medulla. Necrobiotic lesions were present in a few of the animals. In some there were isolated cells or cell cords in the cortex that showed nuclear pyknosis and fragmentation, or coagulation necrosis with complete karyolysis. In a few animals large areas of coagulation and hemorrhagic necrosis were present throughout the cortex. Occasionally hyalin and fibrin thrombi were demonstrable in these areas. In rabbits, the foci of advanced necrosis were usually infiltrated by degenerating and fragmenting polymorphonuclear leucocytes.

Pancreas: There were no significant pathologic changes in 74 specimens examined.

Testes: The 33 specimens examined showed no lesions that could be assigned to the toxin.

Ovary: The 21 specimens examined showed no changes that could be assigned to the toxin.

Urinary bladder: The urinary bladder of 2 guinea pigs showed moderate submucous edema and interstitial hemorrhage. This lesion was identical with lesions seen in guinea pigs on vitamin C deficient diets.

Skeletal muscle: There were no demonstrable lesions.

Brain and spinal cord: The brain and cord were examined in 10 mice, 5 guinea pigs, 5 rabbits, and 3 rats that had received large doses of toxin and survived over 50 hours. Significant lesions were not demonstrable in these specimens.

Lungs: About 12 percent of the guinea pigs and rabbits and a few of the mice and rats showed significant lesions. These consisted of intense vascular engorgement, serous exudate in aggregates of alveoli, diapedesis of variable numbers of red blood cells into scattered alveoli, and occasionally frank alveolar hemorrhages. Necrobiotic lesions were not found.

Bone marrow: The vertebral or sternal marrow of 65 animals was studied after decalcification. Animals that survived over 50 hours showed moderate grades of edema, small scattered focal hemorrhages, and pyknosis of scattered cells or groups of cells. In one guinea pig that lived 59 hours the sternal marrow showed advanced necrosis of large aggregates of marrow cells.

Thymus gland: Edema and congestion were present in 5 of the 11 specimens examined.

PATHOLOGICAL CHANGES IN MICE, RATS, GUINEA PIGS, AND RABBITS PRODUCED BY THE SUBCUTANEOUS AND INTRAMUSCULAR INOCULATION OF TOXIN

Local lesion.—Mice were given from 0.025 to 0.2 cc. and the other animals 0.25 to 2 cc. of the toxin. Mice and rats were resistant to the action of the toxin; rabbits and guinea pigs were moderately susceptible. With the smaller subcutaneous doses, mice and rats showed no reaction or slight swelling, redness, and loss of hair over the area of inoculation after 2 to 5 days. With the largest doses the reaction was more marked and in a small percentage of the animals there was hemorrhagic discoloration of the skin. Incision of the skin after various intervals showed more or less hemorrhagic edema of the subcutis. The process did not extend into the underlying muscle and there was no sloughing of the skin.

Guinea pigs and rabbits usually showed redness of the skin and moderate swelling 2 to 4 days after inoculation of the toxin. With the largest doses the skin frequently showed hemorrhagic discoloration and more or less sloughing after 4 to 7 days.

Deep intramuscular inoculation of large doses of toxin into mice and rats produced slight to moderate swelling of the part, redness of the skin, and loss of hair in about half the group inoculated. The reaction usually reached its height in 3 to 6 days and then subsided. Incision of the part at the height of the reaction showed edema and congestion of the subcutis, and edema, softening, and redness of the muscle. Minor degrees of myolysis were occasionally present.

With the largest doses, guinea pigs and rabbits usually showed spreading boggy edema of the part and purplish discoloration of the skin in 3 to 4 days. Fluctuation of the swelling rapidly developed and in the majority of animals the skin broke by the fifth or sixth day and beet-soup-like fluid escaped. After this fluid escaped, most of the animals recovered. In some, however, the skin did not break until the swelling involved most of the limb and a considerable part of the abdomen. Spontaneous rupture usually occurred on the sixth or seventh day with the escape of the digested fluid tissue. In these cases the bones were frequently exposed. Evisceration occurred in several animals due to liquefaction necrosis of the abdominal wall. In several animals spontaneous amputation at the hip or knee joint occurred. However, the animals showed only slight systemic reaction until death.

Microscopic examination.—Animals were sacrificed at various intervals in order to study the evolution of the local lesion.

Three hours after subcutaneous inoculation of the toxin there was usually marked edema of the entire subcutis. After 8 hours the interstices were greatly enlarged and the subcutaneous pattern was entirely lost, the fibers being separated and torn apart. The epidermis was undermined by fluid and separated from the dermis; glands, blood vessels, and nerves were separated from all supporting tissue and appeared to "float" free in the fluid. Islets of adipose tissue were pushed apart. In some areas small numbers of blood cells were seen, but in others frank hemorrhages were present. The larger blood vessels showed edema of the wall with more or less separation of its elements. Nerves showed interstitial edema.

After 24 hours the epidermis showed hydropic degeneration and dissociation of the epithelial cells. The collagen and elastic fibrils showed swelling, fusion, and pseudocolloid degeneration. The glands showed hydropic degeneration of the cytoplasm of the cells, karyolysis, and desquamation. Here and there the wall of blood vessels showed moderate to marked hydropic degeneration and loss of nuclear staining of the cell structures. Beginning infiltrations of polymorphonuclear leucocytes were present in some areas of edema. This was more prominent in guinea pigs and rabbits. Actual thrombosis of blood vessels was rarely seen.

The essential lesion was the rapidly developing edema. This apparently is an attempt on the part of nature to dilute the intensely irritating and proteolytic effect of the toxin.

Following intramuscular inoculation a similar dissecting and disorganizing edema develops. In the first few hours the process extends along the muscle planes, later the muscle bundles are separated, and finally the individual fibers show irregular swelling and deformity, loss of transverse striation, and changes or complete loss in staining quality. In some bundles only vacuoles are present where there were fibers, and individual fibers often show segmental vacuolation. Despite these changes in the muscle cells the nuclei rarely showed significant alteration.

Myolysis progresses with relatively little associated hemorrhage for the first 24 hours, but later there is considerable hemorrhage and liberation of blood pigment. Moderate infiltrations of polymorphonuclear leucocytes occur as myolysis proceeds.

The smaller blood vessels showed hydropic and hyaline degeneration of the wall, but large vessels rarely showed pathologic changes.

After subcutaneous inoculation of the toxin, only an occasional guinea pig showed minor degenerative changes in the spleen and kidneys.

After intramuscular inoculation, a few of the mice showed degenerative changes in the spleen. None of the rats showed lesions. The guinea pigs and rabbits that showed advanced and extensive local

lesions showed minor to moderate degenerative changes in the spleen, kidneys, and lungs. Pulmonary edema and pulmonary hemorrhages occurred more frequently in this group of animals than in those inoculated intravenously.

SUMMARY

The anaerobe *Clostridium histolyticum* produces a powerful proteolytic toxin and hemolysin. Rats and mice are relatively resistant to the action of the toxin, while guinea pigs and rabbits are moderately susceptible.

Subcutaneous inoculations of the toxin produce only minor changes in rats and mice. Guinea pigs and rabbits usually show redness of the skin and moderate swelling in 2 to 4 days. With large doses there is more or less hemorrhagic discoloration and sloughing.

Deep intramuscular inoculation of large doses of toxin into mice and rats produced minor degrees of swelling and occasionally myolysis. Guinea pigs and rabbits showed a spreading boggy edema, hemorrhagic necrosis of the skin, and severe myolysis. In some animals there was complete denudation of the bone and spontaneous amputation at the hip or knee joint. However, the animals showed only slight systemic reaction.

The essential lesion is a rapidly developing edema that disorganizes and separates the tissues. In the first few hours the edema extends along the muscle planes, later the muscle bundles are separated, and finally the individual fibers show irregular swelling and deformity, loss of transverse striation, and alteration in staining quality. In some bundles only vacuoles are present where there were fibers, and individual fibers often show segmental vacuolation.

Myolysis is associated with more or less hemorrhage and hemolysis. The smaller blood vessels show degenerative changes of the wall, but large ones rarely show lesions.

The variation in survival time of animals receiving approximately the same doses of toxin intravenously afforded an opportunity to observe the effects of the toxin after different time intervals.

Gross lesions were rarely present. Microscopic lesions were found in 56 percent of the mice, 33 percent of the rats, 69 percent of the guinea pigs, and 65 percent of the rabbits.

The spleen showed degenerative changes more regularly than any other organ, and the kidneys were next.

In the spleen the important lesion was pyknosis and karyorrhexis of the lymphocytes in the Malpighian corpuscles. The conversion of follicles into heaps of nuclear debris and phagocytosis of the fragments by reticulum cells was characteristic.

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The kidneys showed granular, hydropic, and colloid droplet degeneration of the tubular epithelium. Occasionally groups of cells or stretches of the epithelium showed coagulation necrosis. Rarely thrombonecrosis of segments of a tuft were present. Interstitial edema and focal hemorrhages were seen in some animals.

In a small number of animals the heart showed moderate interstitial edema and scattered small focal hemorrhages.

Focal necroses in the liver were present in 10 percent of the animals.

The adrenals in an occasional animal showed focal necrosis and hemorrhages.

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NEW CANCER FILM AN IMPORTANT EDUCATIONAL FEATURE

A two-reel sound film illustrating the techniques of modern treatment of cancer by X-rays, radium, and surgery, and dramatizing the saving of life which may be brought about by early diagnosis and treatment, has recently been produced by the United States Public Health Service and the American Society for the Control of Cancer.

The film, entitled "Choose to Live," is an educational feature designed for showing to the general public, and is not only informative but should prove most effective in stimulating persons with suspicious symptoms to seek prompt diagnosis.

The dramatic interest centers in the story of a young mother who fears the symptom which she is concealing from her family but, through dread of what may be revealed, postpones for a time a consultation with her physician. She is prompted to consult her doctor, however, by a lecture on cancer which she hears at her club. In the lecture a doctor tells the story of the fight against cancer, discusses the symptoms which demand attention, and summarizes his talk with the words, "One should not take a chance, for early cancer can be cured."

Mary Brown heeds the warning, consults her family physician, learns that her worst fears are confirmed, and promptly enters the

hospital for an operation. The condition was discovered early, and in a gratifyingly short time Mary returns to her home and family, thankful that she heeded the advice that brought her to her physician. Fortunately for the many prospective victims of the dread disease, "early cancer can be cured."

The film is of high professional standard, produced by skilled technicians, and with the important parts played by professional actors. Interesting shots include the laboratories of the National Institute of Health, 250,000- and 1,000,000-volt X-ray machines in action, the filling of tubes with radon, the use of radium applicators, and other modern procedures. The accompanying musical score is an original arrangement played by members of the National Broadcasting Co. Symphony Orchestra.

The film runs approximately 18 minutes. Prints are available in both 35-mm. and 16-mm. sizes. All have sound recording, which necessitates the use of a projection machine with sound equipment.

A limited number of prints is available on loan from the Public Health Service, the borrower to pay all transportation costs. Local health departments and voluntary health agencies are urged to suggest to their local theater managers that the film be included in their programs. It is suggested that local health departments purchase a copy of the 16-mm. print of the film, if possible, for continued use in their communities. Copies may be purchased from the United States Public Health Service at the actual cost of printing. The prices are $14.68 for the 16-mm. and $32.36 for the 35-mm. print.

All inquiries should be addressed to the Surgeon General, United States Public Health Service, Washington, D. C.

COURT DECISION ON PUBLIC HEALTH

"Sanitary tax" in connection with privies upheld.—(South Carolina Supreme Court; *Town of Marion* v. *Baxley*, 5 S.E.2d 573; decided November 13, 1939.) A regulation of a town board of health provided that every head of a family in the town having in use a sanitary closet or privy should be liable for an annual sanitary tax of $3. This regulation was also adopted as an ordinance of the town, which ordinance made a violation of the regulation punishable by a fine or imprisonment. A resident of the town was convicted for not paying the sanitary tax and he appealed to the supreme court. Such court affirmed the judgment appealed from, and some of the court's conclusions may be briefly stated as follows:

(*a*) The evident purpose of the regulation was to impose an inspection or service charge upon the designated class for the service rendered and privilege permitted, and the constitutional requirement that taxes

levied must be uniform in respect to persons and property had no application.

(*b*) The regulation was valid and in its adoption the board of health acted within the scope of its delegated powers to protect and preserve the public health.

(*c*) In adopting the ordinance the town acted within the scope of the powers delegated to it by the legislature, and the enforcement of the ordinance by fine or imprisonment was not violative of any of the constitutional provisions relied on by the defendant.

DEATHS DURING WEEK ENDED APRIL 13, 1940

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

	Week ended Apr. 13, 1940	Corresponding week, 1939
Data from 88 large cities of the United States:		
Total deaths	8,693	8,852
Average for 3 prior years	8,908	
Total deaths, first 15 weeks of year	140,909	140,992
Deaths under 1 year of age	497	518
Average for 3 prior years	553	
Deaths under 1 year of age, first 15 weeks of year	7,755	8,237
Data from industrial insurance companies:		
Policies in force	65,810,905	67,549,043
Number of death claims	13,144	17,483
Death claims per 1,000 policies in force, annual rate	10.4	13.5
Death claims per 1,000 policies, first 15 weeks of year, annual rate	10.7	11.5

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 APRIL 27, 1940

Summary

The telegraphic reports for the current week reveal what is probably the beginning of the sharp spring rise of Rocky Mountain spotted fever in the western States. Eleven cases were reported in 5 western States, 4 of which occurred in Montana. While these preliminary reports are not complete, the sharp rise suggests that it is time for health officers, in areas in which this disease occurs, to issue precautionary measures for the public. The rise of the seasonal curve and the peak of Rocky Mountain spotted fever incidence occur much earlier in the western States than in the East.

For the week ended April 27, the incidence of each of the 9 important communicable diseases listed in the following table, with the exception of influenza, was below the 5-year (1935–39) median expectancy; and the cumulative totals for the first 17 weeks of the year ended with the current week were below the 5-year medians for the corresponding period for all of the 9 diseases except influenza and poliomyelitis.

As a further indication of the favorable health conditions so far this year, the total number of deaths in 88 large cities for the first 16 weeks ended April 20 was 149,774 as compared with 149,959 in 1939 and with 142,364 in 1938. In 1938, which year recorded the lowest general death rate for the United States, the mortality in large cities was exceptionally low during the month of February.

Telegraphic morbidity reports from State health officers for the week ended April 27, 1940, and comparison with corresponding week of 1939 and 5-year median

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

Division and State	Diphtheria			Influenza			Measles			Meningitis, meningococcus		
	Week ended		Median, 1935–39	Week ended		Median, 1935–39	Week ended		Median, 1935–39	Week ended		Median, 1935–39
	Apr. 27, 1940	Apr. 29, 1939		Apr. 27, 1940	Apr. 29, 1939		Apr. 27, 1940	Apr. 29, 1939		Apr. 27, 1940	Apr. 29, 1939	
NEW ENG.												
Maine	0	1	0	-------	99	6	597	57	72	1	1	0
New Hampshire	0	0	0	-------	-------	-------	15	31	31	0	0	0
Vermont	0	0	0	-------	-------	-------	6	78	78	0	0	0
Massachusetts	3	4	4	-------	-------	-------	118	1,028	667	2	1	3
Rhode Island	3	0	0	-------	-------	-------	188	26	72	0	0	0
Connecticut	0	0	2	1	3	4	41	1,009	464	0	0	0
MID ATL.												
New York	24	17	30	[1]16	[1]25	[1]15	812	1,705	2,927	0	4	13
New Jersey	6	12	16	10	5	8	608	69	1,211	2	0	3
Pennsylvania	28	38	34	-------	-------	-------	330	128	1,113	12	6	6
E. NO. CEN.												
Ohio	7	20	20	54	-------	27	25	32	1,086	1	0	8
Indiana	4	9	9	8	25	21	16	23	332	0	2	2
Illinois	19	43	35	9	60	60	104	36	282	3	2	7
Michigan [2]	2	15	11	14	36	3	674	420	420	0	2	3
Wisconsin	1	0	1	52	115	40	543	765	765	2	0	2
W. NO. CEN.												
Minnesota	0	4	3	-------	6	-------	120	461	382	0	0	0
Iowa	3	7	7	-------	7	6	436	187	187	0	0	1
Missouri	8	2	11	4	1	56	13	16	40	0	2	4
North Dakota	1	1	0	9	26	14	14	45	40	0	0	0
South Dakota	0	1	0	2	9	-------	1	321	5	1	0	0
Nebraska	0	6	5	-------	22	-------	17	622	137	0	0	0
Kansas	8	12	9	8	18	18	630	95	95	0	0	1
SO. ATL.												
Delaware	0	0	0	-------	-------	-------	0	0	17	0	0	0
Maryland [2]	2	2	4	8	18	9	2	348	330	1	1	4
Dist. of Col.	3	4	9	-------	-------	1	1	292	75	0	2	2
Virginia	9	20	12	175	262	-------	184	656	584	0	1	5
West Virginia [2]	14	7	10	55	100	54	15	10	123	3	2	4
North Carolina	4	7	10	14	17	17	135	716	321	0	1	2
South Carolina [2]	4	9	5	270	548	264	12	17	44	0	0	1
Georgia [2]	10	6	6	28	344	53	68	132	0	0	0	1
Florida	2	5	4	9	8	2	99	107	74	0	0	0
E. SO. CEN.												
Kentucky	4	6	10	42	18	16	86	19	405	0	1	10
Tennessee	4	3	4	64	133	59	127	60	60	0	1	6
Alabama [2]	5	8	10	93	545	93	176	178	178	2	1	6
Mississippi	1	5	6	-------	-------	-------	-------	-------	-------	1	0	0
W. SO. CEN.												
Arkansas	4	6	6	92	157	63	30	132	42	0	1	1
Louisiana [2]	8	8	12	12	21	21	12	141	49	0	4	0
Oklahoma	2	2	8	85	107	60	21	186	115	3	1	1
Texas [2]	22	19	31	387	757	479	1,260	406	249	1	2	3
MOUNTAIN												
Montana [4]	1	2	2	4	14	14	40	173	49	0	0	1
Idaho [4]	0	1	0	-------	7	2	87	133	29	0	1	0
Wyoming [4]	3	2	1	1	-------	-------	15	153	42	0	0	0
Colorado [4]	9	9	7	9	14	-------	26	470	356	0	0	0
New Mexico	1	1	3	-------	2	1	30	28	42	0	0	0
Arizona	2	1	2	96	69	36	89	35	54		0	0
Utah [2 4]	1	2	0	10	13	-------	750	93	23		1	0
PACIFIC												
Washington	0	1	1	-------	-------	-------	792	980	327	0	0	0
Oregon [4]	4	2	2	9	44	29	603	75	75	1	0	1
California	11	25	26	68	81	74	397	2,393	1,606	0	0	4
Total	247	355	406	1,718	3,736	1,698	10,315	15,087	15,087	36	40	149
17 weeks	5,970	7,885	9,308	159,244	138,406	126,658	116,620	226,989	226,989	671	854	2,312

See footnotes at end of table.

Telegraphic morbidity reports from State health officers for the week ended April 27, 1940, and comparison with corresponding week of 1939 and 5-year median—Con.

Division and State	Poliomyelitis			Scarlet fever			Smallpox			Typhoid and paratyphoid fever		
	Week ended		Median, 1935–39	Week ended		Median, 1935–39	Week ended		Median, 1935–39	Week ended		Median, 1935–39
	Apr. 27, 1940	Apr. 29, 1939		Apr. 27, 1940	Apr. 29, 1939		Apr. 27, 1940	Apr. 29, 1939		Apr. 27, 1940	Apr. 29, 1939	
NEW ENG.												
Maine	0	0	0	10	12	11	0	0	0	0	1	0
New Hampshire	0	0	0	2	0	6	0	0	0	0	1	1
Vermont	0	0	0	13	7	7	0	0	0	1	0	0
Massachusetts	0	1	0	166	185	246	0	0	0	3	3	2
Rhode Island	0	0	0	6	15	19	0	0	0	0	2	0
Connecticut	0	0	0	119	93	93	0	0	0	3	0	1
MID. ATL.												
New York	2	0	0	977	538	834	0	0	0	9	8	8
New Jersey	0	1	0	396	236	236	0	0	0	6	1	3
Pennsylvania	1	1	1	476	370	539	0	0	0	7	10	9
E. NO. CEN.												
Ohio	1	0	1	505	519	442	0	27	0	3	3	6
Indiana	0	0	0	217	173	173	3	47	10	1	2	1
Illinois	0	1	1	818	483	725	3	25	19	11	3	4
Michigan [2]	3	1	0	326	436	412	4	13	5	4	3	3
Wisconsin	0	1	0	97	184	289	2	0	7	4	2	1
W. NO. CEN.												
Minnesota	0	0	0	72	88	182	3	17	5	1	0	0
Iowa	0	0	0	66	116	166	26	71	40	1	1	2
Missouri	0	1	1	37	73	161	8	18	18	1	2	4
North Dakota	0	0	0	13	12	30	3	7	8	0	0	1
South Dakota	0	0	0	14	15	19	0	7	7	0	0	0
Nebraska	0	0	0	19	54	54	0	8	19	0	0	0
Kansas	0	0	0	75	68	105	0	1	13	1	0	1
SO. ATL.												
Delaware	0	0	0	11	6	6	0	0	0	0	0	0
Maryland [3]	0	0	0	32	48	75	0	0	0	2	0	1
Dist. of Col	0	0	0	30	18	18	0	0	0	0	0	0
Virginia	0	0	0	33	30	30	0	0	0	0	0	5
West Virginia [3]	0	0	0	52	39	41	0	0	0	0	1	3
North Carolina	0	0	0	32	22	23	1	0	1	0	5	2
South Carolina [3]	0	8	1	2	2	2	0	0	0	2	6	4
Georgia [3]	0	3	0	6	5	6	0	0	0	3	1	3
Florida	0	0	0	7	5	5	1	0	0	0	0	2
E. SO. CEN.												
Kentucky	0	0	0	83	42	40	1	1	0	2	2	4
Tennessee	0	1	0	74	53	27	0	6	1	0	0	2
Alabama [3]	0	3	1	12	8	6	1	3	2	4	6	4
Mississippi	1	0	0	9	0	3	0	0	0	0	4	3
W. SO. CEN.												
Arkansas	0	0	0	5	6	6	3	11	4	0	4	1
Louisiana [3]	0	1	1	5	20	11	0	0	1	6	13	13
Oklahoma	0	0	0	12	15	24	1	43	3	0	4	4
Texas [3]	2	0	0	26	37	39	6	13	4	5	8	8
MOUNTAIN												
Montana [4]	0	0	0	29	17	17	0	4	7	1	0	1
Idaho [4]	0	0	0	7	4	9	0	8	5	1	5	1
Wyoming [4]	0	0	0	8	5	7	0	0	2	1	0	0
Colorado [5]	0	0	0	44	34	62	1	0	2	0	0	0
New Mexico	0	0	0	23	22	24	1	4	0	1	0	1
Arizona	0	1	0	6	15	15	0	4	0	1	7	1
Utah [2 4]	0	0	0	13	26	32	2	1	1	0	0	0
PACIFIC												
Washington	0	0	0	41	35	35	0	1	12	0	2	2
Oregon [4]	0	0	0	11	26	32	0	6	16	1	1	1
California	3	4	3	133	169	170	6	17	17	5	1	6
Total	13	28	21	5,170	4,386	6,904	76	363	363	91	112	129
17 weeks	412	292	347	81,757	86,301	117,155	1,235	6,150	5,485	1,346	1,951	1,951

See footnotes at end of table.

Telegraphic morbidity reports from State health officers for the week ended April 27, 1940, and comparison with corresponding week of 1939 and 5-year median—Con.

Division and State	Whooping cough	
	Week ended	
	Apr. 27, 1940	Apr. 29, 1939
NEW ENG.		
Maine	16	24
New Hampshire	40	4
Vermont	0	23
Massachusetts	150	220
Rhode Island	9	93
Connecticut	82	70
MID. ATL.		
New York	332	486
New Jersey	113	278
Pennsylvania	215	354
E. NO. CEN.		
Ohio	257	229
Indiana	37	60
Illinois	114	202
Michigan [2]	196	180
Wisconsin	80	170
W. NO. CEN.		
Minnesota	41	44
Iowa	29	15
Missouri	4	10
North Dakota	16	5
South Dakota	0	4
Nebraska	3	16
Kansas	43	21
SO. ATL.		
Delaware	5	6
Maryland [2]	140	25
Dist. of Col.	22	33
Virginia	31	79
West Virginia [2]	35	18
North Carolina	76	293
South Carolina [3]	20	84
Georgia [3]	5	26
Florida	32	37
E. SO. CEN.		
Kentucky	84	14
Tennessee	32	36
Alabama [3]	18	39
Mississippi	----------	----------
W. SO. CEN.		
Arkansas	33	15
Louisiana [3]	63	23
Oklahoma	9	0
Texas [3]	318	146
MOUNTAIN		
Montana [4]	4	4
Idaho [4]	7	4
Wyoming [4]	0	1
Colorado [5]	16	66
New Mexico	144	42
Arizona	31	15
Utah [3 4]	134	57
PACIFIC		
Washington	81	37
Oregon [4]	20	12
California	455	217
Total	3,542	3,837
17 weeks	51,872	69,070

[1] New York City only.
[2] Period ended earlier than Saturday.
[3] Typhus fever, week ended Apr. 27, 1940, 18 cases as follows: South Carolina, 1; Georgia, 5; Alabama, 4; Louisiana, 2; Texas, 6.
[4] Rocky Mountain spotted fever, week ended Apr. 27, 1940, 11 cases as follows: Montana, 4; Idaho, 1; Wyoming, 2; Utah, 3; Oregon, 1.
[5] Colorado tick fever, week ended Apr. 27, 1940, Colorado, 1 case.

VENEREAL DISEASES

New Cases Reported for February 1940 [1]

Reports from States

	Syphilis										Gonorrhea		Other venereal diseases	
	Early			Late		Congenital		All syphilis [2]						
	Primary and secondary	Early-latent [3]	Rate per 10,000 population	Includes late latent	Rate per 10,000 population	Number	Rate per 10,000 population	Number	Rate per 10,000 population		Number	Rate per 10,000 population	Number	Rate per 10,000 population
Alabama [4]														
Alaska								8			25			
Arizona	36	14	1.20	29	.69	13	0.31	199	4.76		163	3.90	4	0.10
Arkansas	171	149	1.54	204	.98	15	.07	1,074	5.18		181	.87	5	.02
California		[5] 345	.55	1,176	1.88	72	.12	1,682	2.69		1,243	1.99	19	.03
Colorado	30		.28	74	.69	6	.06	110	1.02		60	.56		
Connecticut	15	10	.14	70	.40	7	.04	155	.89		97	.55		
Delaware	4	13	.65	10	.88	9	.34	156	5.93		31	1.18		
District of Columbia								542	8.52		229	3.60	2	.03
Florida	23	370	2.31	865	5.09	59	.85	1,437	8.46		183	1.08	14	.08
Georgia	1,097	692	3.52					1,789	5.75		84	.27	15	.05
Hawaii	4	3	.02	36	.09	3	.01	66	.16		41	.10		
Idaho	12		.24	[6] 37	.74	3	.06	57	1.14		23	.46	2	.04
Illinois	123	351	.60	1,160	1.47	69	.09	1,703	2.15		1,026	1.30	23	.03
Indiana	91	40	.38	216	.62	18	.06	490	1.40		111	.32	1	.002
Iowa [4]														
Kansas	52	29	.43	60	.32	14	.08	231	1.24		75	.40		
Kentucky	107	37	.49	341	1.15	26	.09	623	2.11		252	.85	2	.01
Louisiana	439	1	2.05	1	.01	8	.04	761	3.55		76	.35	18	.08
Maine	10		.12	40	.47	9	.10	59	.69		40	.46	1	.01
Maryland	67	16	.49	131	.78	6	.04	627	3.72		189	1.12	20	.12
Massachusetts	71		.16	[6] 311	.70	23	.05	405	.91		291	.66		
Michigan	96	114	.43	302	.62	35	.07	720	1.48		499	1.02	20	.04
Minnesota	12	15	.11	178	.67	9	.03	214	.80		231	.86		
Mississippi	262	673	4.58	529	2.59	56	.27	3,679	18.03		2,363	11.58		
Missouri								235	.58		30	.07	1	.002
Montana	10		.18	18	.33	1	.02	36	.66		26	.48		
Nebraska	18	5	.17	28	.21	2	.01	53	.39		45	.33		
Nevada	8		.30	20	1.96			23	2.25		11	1.08		
New Hampshire				11	.21	2	.04	19	.37		8	.15		
New Jersey	107	114	.51	470	1.08	54	.12	855	1.96		221	.51	4	.01
New Mexico	354	242	.46	1,167	.90	1,743	1.34	3,669	2.82		1,451	1.12	27	.02
New York	98		.08	925	.72	64	.05	1,097	.84		341	.26		
North Carolina	193	846	2.94	728	2.06	61	.17	1,828	5.18		329	.93	29	.08
North Dakota	3	7	.14	10	.14	2	.03	27	.38		29	.41		
Ohio	176	213	.58	626	.93	43	.06	1,058	1.57		328	.49	20	.03
Oklahoma	390	719	4.32	1,416	5.51	231	.90	3,496	13.60		50	.19		
Oregon	27	27	.52	79	.76	7	.07	145	1.40		96	.92		
Pennsylvania [4]														
Rhode Island	6		.09	68	1.00	5	.07	105	1.54		51	.75		
South Carolina	619	549	6.17	789	4.17	57	.30	2,058	10.88		52	.27	6	.03
South Dakota	4	8	.17	19	.27	4	.06	36	.52		15	.22		
Tennessee	179	364	1.86	410	1.40	25	.09	978	3.34		307	1.05	12	.04
Texas	310	405	1.15	764	1.23	112	.18	2,111	3.39		870	1.40	62	1.00
Utah	15	4	.36	46	.88	5	.10	70	1.34		36	.69		
Vermont	7	8	.39	9	.23			24	.62		10	.26		
Virginia	301	208	1.86	699	2.55	56	.20	1,375	5.01		201	.73		
Washington	61	37	.59	127	.76	9	.05	260	1.55		289	1.73		
West Virginia								208	1.09		87	.46		
Wisconsin	29		.10	164	.56	3	.01	196	.67		48	.16		
Wyoming	4	5	.38	16	.68	3	.13	33	1.39		11	.46	1	.04
Puerto Rico [4]														
Virgin Islands [4]														
Total	5,558	6,633	1.13	13,558	1.26	2,891	.27	35,815	3.11		12,136	1.06	308	.05

See footnotes at end of table.

Reports from cities of 200,000 population or over

	Syphilis									Gonorrhea		Other venereal diseases	
	Early			Late		Congenital		All syphilis [2]					
	Primary and secondary	Early-latent	Rate per 10,000 population	Includes late latent	Rate per 10,000 population	Number	Rate per 10,000 population	Number	Rate per 10,000 population	Number	Rate per 10,000 population	Number	Rate per 10,000 population
Akron	15	8	.84	16	.58	7	.25	46	1.67	22	.80	1	.04
Atlanta	----	184	6.13	4	.13	----	----	188	6.26	22	.73	1	.03
Baltimore	57	10	.80	112	1.34	1	.01	385	4.61	113	1.35	17	.20
Birmingham	57	26	2.82	121	4.11	13	.44	310	10.53	47	1.60	3	.10
Boston	24	7	.39	92	1.16	6	.08	154	1.94	----	----	----	----
Buffalo	19	----	.32	102	1.70	----	----	121	2.01	42	.70	----	----
Chicago	72	157	6.24	749	2.04	40	1.09	1,018	2.78	680	1.86	23	.63
Cincinnati [4]	----	----	----	----	----	----	----	----	----	----	----	----	----
Cleveland	31	33	.68	139	1.47	7	.07	210	2.22	85	.90	8	.08
Columbus	14	12	.83	31	.99	5	.16	62	1.98	51	1.63	----	----
Dallas	49	54	3.39	137	4.51	1	.03	241	7.93	143	4.70	15	.49
Dayton	5	3	.36	28	1.26	2	.09	38	1.71	26	1.17	----	----
Denver	----	----	----	----	----	----	----	134	4.44	80	2.66	----	----
Detroit	54	67	6.66	256	1.41	14	.07	391	2.15	293	1.61	23	1.27
Houston	31	57	2.46	130	3.63	11	.31	321	8.96	107	2.99	4	.11
Indianapolis	14	1	.39	23	.60	2	.05	97	2.52	38	.99	----	----
Jersey City	6	14	.62	20	.62	2	.06	42	1.29	4	.12	2	.06
Kansas City [4]	----	----	----	----	----	----	----	----	----	----	----	----	----
Los Angeles	----	119	.78	348	2.29	20	.14	487	3.20	324	2.13	5	.03
Louisville	19	6	.74	118	3.48	9	.27	180	5.31	67	1.98	3	.09
Memphis [4]	----	----	----	----	----	----	----	----	----	----	----	----	----
Milwaukee	7	----	.11	131	2.08	----	----	138	2.19	14	.22	14	.22
Minneapolis	5	6	.22	38	.76	1	.02	50	1.00	68	1.36	----	----
Newark	21	5	.57	178	3.92	6	.13	210	4.62	68	1.50	----	----
New Orleans	----	----	----	----	----	----	----	81	1.66	45	.92	12	.25
New York	256	242	.66	1,679	2.24	94	.12	2,572	3.43	1,105	1.47	32	.04
Oakland	4	18	.70	86	2.75	2	.06	110	3.51	82	2.62	1	.03
Omaha	6	10	.72	10	.45	1	.04	27	1.21	19	.85	----	----
Philadelphia [4]	----	----	----	----	----	----	----	----	----	----	----	----	----
Pittsburgh	----	----	----	----	----	----	----	349	4.95	14	.20	----	----
Portland [4]	----	----	----	----	----	----	----	----	----	----	----	----	----
Providence [4]	----	----	----	----	----	----	----	----	----	----	----	----	----
Rochester	3	----	.09	[7] 31	.90	----	----	34	.99	35	1.02	----	----
St. Louis	99	190	3.43	483	5.73	25	.30	797	9.45	137	1.63	4	.05
St. Paul [4]	----	----	----	----	----	----	----	----	----	----	----	----	----
San Antonio	134	325	17.55	75	2.87	7	.27	539	20.60	49	1.87	1	.04
San Francisco	61	----	.89	170	2.47	7	.11	238	3.45	180	2.61	6	.09
Seattle	14	26	1.03	57	1.47	6	.15	157	4.06	147	3.80	1	.03
Syracuse	1	1	.09	84	3.73	6	.27	92	4.08	8	.36	----	----
Toledo	7	2	.29	48	1.54	3	.10	60	1.93	16	.51	3	.10
Washington	----	----	----	----	----	----	----	542	8.52	229	3.60	2	.03
Total	1,086	1,583	1.03	5,498	2.12	298	.11	10,424	3.71	4,385	1.01	181	.08

[1] Figures preliminary and subject to correction.
[2] Includes "not stated" diagnosis.
[3] Duration of infection under 4 years.
[4] No report for current month.
[5] Breakdown for primary, secondary, and early latent not available.
[6] Includes early latent, late, and late latent.
[7] Includes early latent.

WEEKLY REPORTS FROM CITIES

City reports for week ended April 13, 1940

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.

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								
Data for 90 cities:											
5-year average	142	279	81	7,641	759	2,340	24	406	21	1,242	----
Current week [1]	51	154	42	2,322	506	2,031	8	340	17	1,025	----
Maine:											
Portland	0	----	0	150	2	2	0	0	0	5	17
New Hampshire:											
Concord	0	----	0	0	2	0	0	0	0	0	12
Manchester	0	----	0	5	0	0	0	0	0	0	13
Nashua	0	----	0	5	1	0	0	0	0	0	8
Vermont:											
Barre	----	----	----	----	----	----	----	----	----	----	----
Burlington	0	----	0	0	0	0	0	0	0	0	8
Rutland	0	----	0	0	1	0	0	0	0	0	5
Massachusetts:											
Boston	0	----	0	104	37	74	0	8	0	57	255
Fall River	3	----	0	42	2	1	0	1	0	8	36
Springfield	0	----	0	3	1	6	0	0	0	5	28
Worcester	0	----	0	4	4	12	0	1	0	6	55
Rhode Island:											
Pawtucket	0	----	0	1	0	1	0	0	0	0	19
Providence	2	----	0	103	6	6	0	0	0	6	68
Connecticut:											
Bridgeport	0	2	2	2	3	3	0	1	0	0	44
Hartford	0	----	0	0	2	6	0	0	0	2	61
New Haven	0	3	2	0	3	5	0	1	0	0	42
New York:											
Buffalo	1	----	1	1	8	7	0	4	0	8	128
New York	8	14	3	127	99	717	0	77	4	118	1,559
Rochester	0	----	0	12	2	10	0	2	0	7	77
Syracuse	0	----	0	0	3	13	0	1	0	1	44
New Jersey:											
Camden	0	----	0	0	1	9	0	0	1	0	27
Newark	0	2	0	300	4	28	0	3	1	32	106
Trenton	0	----	0	0	5	5	0	2	0	0	44
Pennsylvania:											
Philadelphia	1	3	1	71	25	116	0	20	1	54	491
Pittsburgh	0	----	2	1	17	28	0	11	1	6	161
Reading	1	----	0	0	0	0	0	1	1	4	16
Scranton	0	----	----	0	----	6	0	----	1	0	----
Ohio:											
Cincinnati	0	1	1	3	9	4	0	12	0	19	167
Cleveland	0	24	2	0	7	47	0	7	0	26	165
Columbus	0	2	2	1	2	14	0	3	0	14	92
Toledo	0	1	1	1	5	35	0	1	0	18	73
Indiana:											
Anderson	0	----	0	0	2	2	0	0	0	1	12
Fort Wayne	0	----	0	1	0	2	0	3	0	0	26
Indianapolis	0	----	1	1	4	10	0	2	0	8	107
Muncie	0	----	0	0	0	0	0	0	0	0	10
South Bend	0	----	0	0	1	0	0	0	0	1	16
Terre Haute	1	----	2	0	2	1	0	1	0	1	28
Illinois:											
Alton	0	----	0	0	1	0	0	0	0	6	12
Chicago	7	2	3	52	39	526	0	40	0	49	744
Elgin	0	----	0	0	2	1	0	0	0	0	16
Moline	1	----	0	5	0	2	0	0	0	0	10
Springfield	0	----	0	0	3	3	0	0	0	2	16
Michigan:											
Detroit	1	2	0	46	12	61	0	15	1	44	265
Flint	0	----	0	1	8	16	0	2	0	11	35
Grand Rapids	0	----	0	3	7	32	0	0	0	14	42
Wisconsin:											
Kenosha	0	----	0	74	1	4	0	0	0	0	12
Madison	0	----	0	1	1	3	0	0	0	5	17
Milwaukee	0	1	1	22	9	20	0	4	0	8	87
Racine	0	----	0	2	0	5	0	0	0	0	13
Superior	0	----	0	62	0	4	0	0	0	0	6

[1] Figures for Barre estimated; report not received.

City reports for week ended April 13, 1940—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								
Minnesota:											
Duluth	0	------	1	63	1	6	0	0	0	0	28
Minneapolis	2	------	0	3	6	17	1	2	0	7	108
St. Paul	0	------	0	2	1	6	0	0	0	3	46
Iowa:											
Cedar Rapids	0	------	--------	33	-------	3	0	-------	0	1	--------
Davenport	0	------	--------	17	-------	4	0	-------	0	0	--------
Des Moines	0	------	--------	7	-------	8	1	-------	0	0	43
Sioux City	0	------	--------	0	-------	1	0	-------	0	0	--------
Waterloo	0	------	--------	2	-------	2	0	-------	0	0	--------
Missouri:											
Kansas City	0	------	0	9	6	12	0	6	0	0	84
St. Joseph	0	------	0	0	2	0	0	1	0	1	31
St. Louis	2	------	0	2	16	21	0	6	0	5	165
North Dakota:											
Fargo	0	------	0	0	0	0	0	0	0	0	7
Grand Forks	0	------	--------	0	-------	0	0	-------	0	1	--------
Minot	1	------	0	0	0	0	0	0	0	0	4
South Dakota:											
Aberdeen	0	------	--------	0	-------	1	0	-------	0	2	--------
Sioux Falls	0	------	0	0	0	7	0	0	0	0	5
Nebraska:											
Lincoln	0	------	--------	1	-------	3	0	-------	0	0	--------
Omaha	0	------	0	15	4	2	0	1	0	3	51
Kansas:											
Lawrence	0	5	0	0	1	0	0	0	0	0	9
Topeka	0	------	0	2	1	2	0	0	0	0	9
Wichita	0	------	0	79	5	2	0	0	0	2	32
Delaware:											
Wilmington	0	------	0	0	3	3	0	0	0	0	27
Maryland:											
Baltimore	1	5	1	1	25	14	0	8	1	163	233
Cumberland	0	------	0	0	0	1	0	0	0	0	13
Frederick	0	------	0	0	1	1	0	0	0	0	5
District of Columbia:											
Washington	2	1	1	3	16	25	0	6	0	10	178
Virginia:											
Lynchburg	0	------	0	2	0	0	0	0	0	11	7
Norfolk	0	17	0	0	4	4	0	0	0	0	27
Richmond	0	------	1	1	2	2	0	6	1	2	54
Roanoke	0	------	0	1	1	4	0	0	0	4	17
West Virginia:											
Charleston	0	2	1	0	5	0	0	0	0	0	28
Huntington	0	------	--------	0	-------	1	0	-------	0	0	--------
Wheeling	0	------	--------	0	-------	0	0	-------	0	0	--------
North Carolina:											
Gastonia	0	------	--------	0	-------	1	0	0	-------	0	--------
Raleigh	0	------	0	0	0	0	0	1	0	2	10
Wilmington	0	------	0	0	0	0	0	1	0	0	8
Winston-Salem	1	1	0	0	3	0	0	1	0	2	26
South Carolina:											
Charleston	1	35	0	0	2	0	0	0	0	0	13
Florence	0	------	0	0	0	0	0	0	0	0	11
Greenville	0	------	0	0	11	0	0	1	0	0	31
Georgia:											
Atlanta	1	9	0	15	0	8	0	5	0	4	95
Brunswick	0	------	0	0	0	0	0	0	1	0	1
Savannah	0	9	1	0	3	0	0	1	0	0	31
Florida:											
Miami	0	2	0	2	3	1	0	1	0	0	44
Tampa	0	2	2	61	0	0	0	1	0	0	21
Kentucky:											
Ashland	0	------	0	0	0	0	0	0	0	2	3
Covington	0	------	0	0	2	0	0	0	1	5	9
Lexington	0	------	0	3	1	2	0	4	0	8	17
Louisville	0	3	0	2	5	24	0	0	0	62	38
Tennessee:											
Knoxville	0	------	0	0	3	8	0	1	0	0	27
Memphis	0	------	2	33	7	16	6	3	0	12	86
Nashville	0	------	1	1	3	0	0	2	0	4	31
Alabama:											
Birmingham	0	3	0	10	5	1	0	3	0	1	61
Mobile	0	4	1	17	0	0	0	2	0	3	23
Montgomery	0	2	--------	9	-------	0	0	-------	0	1	--------

City reports for week ended April 13, 1940—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								
Arkansas:											
Fort Smith	0	1		0		0	0		0	0	
Little Rock	0		0	0	3	0	0	0	0	0	
Louisiana:											
Lake Charles	0		0	1	0	0	0	0	0	0	3
New Orleans	2	2	1	5	13	6	0	9	0	40	144
Shreveport	0		0	0	3	0	0	0	0	0	46
Oklahoma:											
Oklahoma City	0	4	0	0	4	0	0	1	0	2	44
Tulsa	0			2		3	0		0	14	
Texas:											
Dallas	3		1	119	2	1	0	3	2	26	58
Fort Worth	0		2	3	2	4	0	3	1	21	49
Galveston	0		0	0	2	1	0	0	0	0	15
Houston	1	1	1	8	3	1	0	6	0	1	81
San Antonio	0	7	2	30	3	1	0	7	0	9	72
Montana:											
Billings	0		0	0	1	0	0	0	0	0	12
Great Falls	0		0	0	0	0	0	0	0	3	4
Helena	0		0	0	0	1	0	0	0	0	2
Missoula	0		0	0	2	0	0	0	0	0	7
Idaho:											
Boise	0		0	0	0	0	0	0	0	1	10
Colorado:											
Colorado Springs	0		0	0	4	2	0	2	0	0	20
Denver	3		0	11	2	6	0	6	0	4	78
Pueblo	0		0	3	0	5	0	1	0	0	11
New Mexico:											
Albuquerque	0			0		0	0		0	17	
Utah:											
Salt Lake City	0		0	216	1	5	1	1	0	65	34
Washington:											
Seattle	1		0	389	1	2	0	6	1	39	100
Spokane	0		0	2	0	3	0	0	1	10	27
Tacoma	0		0	3	3	7	0	0	0	1	34
Oregon:											
Portland	2		0	210	1	4	0	3	0	9	74
Salem	0			4		0	0		0	1	
California:											
Los Angeles	3	13	0	16	6	34	0	11	0	28	362
Sacramento	2	1	0	9	5	4	0	2	0	26	33
San Francisco	1	3	2	3	8	12	0	10	0	17	179

State and city	Meningitis, meningococcus		Poliomyelitis cases	State and city	Meningitis, meningococcus		Poliomyelitis cases
	Cases	Deaths			Cases	Deaths	
New York:				**District of Columbia:**			
New York	3	0	1	Washington	0	1	0
Syracuse	1	0	0	**West Virginia:**			
Ohio:				Wheeling	1	0	0
Cleveland	1	0	0	**Texas:**			
Toledo	0	0	1	Dallas	1	0	0
Indiana:				Fort Worth	0	0	1
South Bend	0	1	0	**California:**			
Illinois:				Los Angeles	0	0	1
Chicago	1	0	0				
Maryland:							
Baltimore	1	0	0				

Encephalitis, epidemic or lethargic.—Cases: New York, 3; Rochester, 1; Pittsburgh, 1; Columbus, 1; Great Falls, 1.

Pellagra.—Cases: Savannah, 2; Memphis, 2; Birmingham, 1; Dallas, 1; San Francisco, 3.

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

FOREIGN REPORTS

CANADA

Provinces—Communicable diseases—Week ended March 30, 1940.—During the week ended March 30, 1940, 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	4	3				1	9
Chickenpox		14		184	374	30	5	15	53	675
Diphtheria			2	16	2	2	1	1		24
Dysentery				90	1					91
Influenza		15			55		10		2	82
Measles			1	136	618	624	141	5	67	1,592
Mumps				32	254	9	126	3	7	431
Pneumonia	7	4			24	6	2		5	48
Poliomyelitis						1	1			2
Scarlet fever		6	5	88	126	9	3	23	4	264
Trachoma						1			3	4
Tuberculosis		36	15	54	56			1		162
Typhoid and paratyphoid fever				26	2	36			1	65
Whooping cough	2		4	97	97	18	20	4	21	263

FINLAND

Communicable diseases—4 weeks ended February 24, 1940.—During the 4 weeks ended February 24, 1940, cases of certain communicable diseases were reported in Finland as follows:

Disease	Cases	Disease	Cases
Diphtheria	251	Poliomyelitis	6
Dysentery	2	Scarlet fever	475
Influenza	2,546	Typhoid fever	25
Paratyphoid fever	29	Undulant fever	1

JAMAICA

Communicable diseases—4 weeks ended January 20, 1940.—During the 4 weeks ended January 20, 1940, cases of certain communicable diseases were reported in Kingston, Jamaica, and in the island outside of Kingston, as follows:

Disease	Kingston	Other localities	Disease	Kingston	Other localities
Chickenpox		11	Puerperal sepsis		2
Diphtheria		2	Tuberculosis	29	73
Dysentery	12	3	Typhoid fever	6	42
Leprosy		3			

SCOTLAND

Vital statistics—Fourth quarter 1939.—Following are vital statistics for Scotland for the fourth quarter of 1939:

	Number	Rate per 1,000 population		Number	Rate per 1,000 population
Marriages	13,015	10.3	Deaths from—Continued.		
Births	20,306	16.1	Homicide	6	
Deaths	16,159	12.8	Influenza	87	.07
Deaths under 1 year	1,403	[1] 69	Lethargic encephalitis	14	
Deaths from:			Measles	5	
Appendicitis	100		Nephritis, acute and chronic	331	
Cancer	2,005	1.59	Pneumonia (all forms)	640	.51
Cerebral hemorrhage and apoplexy	1,130		Poliomyelitis	3	
Cerebrospinal fever	18	.01	Puerperal sepsis	14	
Cirrhosis of the liver	47		Scarlet fever	8	.01
Diabetes mellitus	211		Senility	631	
Diarrhea and enteritis (under 2 years)	237		Suicide	90	
Diphtheria	129	.10	Syphilis	19	
Dysentery	13		Tetanus	2	
Erysipelas	12		Tuberculosis (all forms)	806	.64
Heart disease	4,094		Typhoid fever and paratyphoid fever	5	
			Whooping cough	32	.03

[1] Per 1,000 live births.

Vital statistics—Year 1939.—Following are vital statistics for Scotland for the year 1939:

	Number	Rate per 1,000 population		Number	Rate per 1,000 population
Marriages	46,257	9.2	Deaths from—Continued.		
Live births	86,899	17.4	Heart disease	15,458	
Deaths	64,413	12.9	Influenza	915	
Deaths under 1 year	5,955	[1] 69	Measles	15	
Deaths from:			Nephritis, acute and chronic	1,756	
Appendicitis	387		Pneumonia (all forms)	2,949	.59
Cancer	8,040	1.61	Puerperal sepsis	88	
Cerebral hemorrhage and apoplexy	6,760		Scarlet fever	47	
Cirrhosis of the liver	179		Suicide	458	
Diabetes mellitus	819		Tuberculosis (all forms)	3,526	.70
Diarrhea and enteritis (under 2 years)	1,041		Typhoid fever and paratyphoid fever	26	
Diphtheria	395		Whooping cough	397	

[1] Per 1,000 live births.

VIRGIN ISLANDS

Notifiable diseases—January–March 1940.—During the months of January, February, and March 1940, cases of certain notifiable diseases were reported in the Virgin Islands as follows:

Disease	January	February	March	Disease	January	February	March
Cerebrospinal meningitis		1		Pneumonia		1	
Chickenpox		1	1	Schistosomiasis		1	
Filariasis	11	5	10	Syphilis	17	19	18
Gonorrhea	19	10	13	Tetanus	1		1
Hookworm disease	4	6	3	Trachoma		3	
Malaria	2	1		Tuberculosis	1	1	1
Pellagra		1		Typhoid fever		1	

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

NOTE.—A cumulative table giving current information regarding the world prevalence of quarantinable diseases appeared in the PUBLIC HEALTH REPORTS of April 26, 1940, pages 745–749. A similar table will appear in future issues of the PUBLIC HEALTH REPORTS for the last Friday of each month.

Plague

Argentina—Salta Province—Isla (vicinity of).—For the period March 16–31, 1940, 1 case of plague with 1 death was reported in the vicinity of Isla, Salta Province, Argentina.

Hawaii Territory—Island of Hawaii—Hamakua District—Hamakua Mill Area.—A rat found on March 25, 1940, in Hamakua Mill Area, Hamakua District, Island of Hawaii, T. H., has been proved positive for plague.

Smallpox

Thailand—Bangkok.—During the week ended April 6, 1940, 2 cases of smallpox were reported in Bangkok, Thailand.

Yellow Fever

Colombia—Caldas Department—Samana.—On March 23, 1940, 1 death from yellow fever was reported in Samana, Caldas Department, Colombia.

Gold Coast—Prestea (vicinity of).—On April 6, 1940, 1 fatal case of yellow fever was reported in the vicinity of Prestea, Gold Coast.

Nigeria—Ogoja Region—Enugu.—On April 1, 1940, 1 suspected case of yellow fever was reported in Enugu, Ogoja Region, Nigeria.