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EDITORIAL

ANTIBIOTICS—THE NEW WEAPON IN TUBERCULOSIS

Since the discovery of the tubercle bacillus by Koch in 1882, repeated and persistent efforts have been made to find a drug or antibiotic that would be effective in the cure of tuberculosis. Men of science in almost every nation of the world have worked through lifetimes to find a lethal agent to defeat a germ that has consistently resisted every attempt against its predatory existence. Over the years, the hopes of the ill have been lifted by such attempts at treatment as tuberculin injections, gold therapy, the application of sulpha drugs, and various vaccines. In every instance the high hopes were dashed by failure. Although investigations continued, few drug cures for tuberculosis were offered until very recently, when Waksman isolated a promising compound—streptomycin—from certain species of the soil actinomycetes. Streptomycin has forged ahead, and, in laboratory and animal trials, has become the current drug of promise. At the moment, streptomycin is being tried on human beings and, although no extensive controlled experiments have been performed, preliminary results not only give hope of suppressive action, even in meningitis and miliary tuberculosis, but also point the way to further investigation and search for similar antibiotics that may be even safer and more economical.

It should be pointed out that penicillin, although not effective against tuberculosis, has been largely responsible for vigorous research into antibacterial substances in the soil. Here, indeed, is a vast field for scientific effort and ingenuity. Individual workers and teams of scientists should apply their separate and collective talents to this field, where the deadly enemy of the tubercle bacilli may be lying, not too obscurely, in hiding.

This is the eleventh of a series of special issues of PUBLIC HEALTH REPORTS devoted exclusively to tuberculosis control, which will appear the first week of each month. The series began with the Mar. 1, 1946 issue. The articles in these special issues are reprinted as extracts from the PUBLIC HEALTH REPORTS. Effective with the July 5 issue, these extracts may be purchased from the Superintendent of Documents, Government Printing Office, Washington 25, D. C., for 10 cents a single copy. Subscriptions are obtainable at $1.00 per year; $1.25 foreign.

Thus far, in the field of vaccine therapy, only BCG vaccine has proved to be an effective adjunct to conventional tuberculosis control methods. Such application, however, is limited to uninfected persons, and there are still objections to the use of live vaccine in the United States. BCG has been found to be beneficial as a control measure particularly in the Scandinavian and South American countries, and when its effectiveness can be prolonged, and killed organisms employed, it will be even more important in control, especially where exposure rates are high and treatment facilities poor.

The central problem still remains. We must discover a specific drug or antibiotic that will prevent and cure tuberculosis. However, we must observe certain precautions and take guarded care every step of the way. Moreover, the drug that eventually will be used should properly have definite characteristics, because tuberculosis is a long-term disease and repeated dosage of any drug will probably be necessary. Any drug ultimately used must be reasonable in cost, abundant in nature, or susceptible to simple and economical manufacture. Purity of the drug will need to be carefully determined. Recent studies with penicillin have demonstrated many variants of low potency. Development of resistant strains of tubercle bacilli must be watched for, especially in a disease which requires long periods of treatment during which the disease organism may achieve tolerance for the drug.

It should be kept in mind that any antibiotic, even though effective against the tubercle bacilli, may be of little benefit to far-advanced cases, because irreversible processes have set in and, in most instances, the blood supply to areas of cavitation and other areas with extensive involvement has been cut off. The drug, therefore, cannot be carried to those areas and may only prevent spread in surrounding tissues. For this reason, we must not expect too much of any drug, no matter how effective it may be in early cases of tuberculosis.

To be wholly effective, we must couple the use of an antibiotic with sound case-finding techniques, so as to discover early cases and to treat them at once. In this connection, additional research should go forward to discover some laboratory method, such as a complement-fixation test, for the diagnosis of tuberculosis when careful search does not reveal positive bacillary findings although the tuberculin test and X-ray findings are positive. Since many chest lesions are nontuberculous, such a laboratory method would be an essential companion to an antibiotic.

Often in the past, in the treatment of tuberculosis, promising drugs have been applied prematurely to human beings. It should continue to be the practice to subject any substance to exhaustive test-tube and animal experimentation and to make careful trials of its safety

and effectiveness, before controlled studies on human beings are undertaken.

The leading article in this issue, "A Crystalline Antibacterial Substance from the Lichen *Ramalina reticulata*," is another example of the careful laboratory and animal work that so much needs to be done in this field. It is hoped that from such beginnings, work of widespread scope will be undertaken on animals and, if justified, on human beings later. It is through such studies as this that one by one the antibacterial possibilities in tuberculosis are tested. Cumulatively, such research enterprise creates a decisive weapon for the final victory over tuberculosis.

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A CRYSTALLINE ANTIBACTERIAL SUBSTANCE FROM THE LICHEN *RAMALINA RETICULATA*

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Ramalina reticulata (Noedh.) Kremph., sometimes called California Spanish moss, is a lichen of the Family Usneaceae which grows as an epiphyte along the west coast of North America from California to Alaska (*1*). The plant has no integument but does contain in the interstices between hyphae and algal cells a carbohydrate substance which is very hygroscopic, so that under foggy conditions it is soft, friable, and saturated with water. During the foggy season, the plant may remain water-soaked for long periods of time. The carbohydrate, when separated from the plant, is an excellent medium for the growth of many types of bacteria. These conditions suggest the presence of a chemical substance in the lichen which inhibits the growth of bacteria.

A few simple observations were made which supported this inference:

1. By boiling the lichen in water and then cooling, a gelatinous carbohydrate material was obtained. A suspension of this material in sterile water was exposed to air and was found, in a few days, to be teeming with bacteria.

2. When strands of fresh lichen were placed on nutrient agar with sterile forceps and incubated at 25° C., no bacterial colonies were found, although occasional fungi of several types grew out from the surface or ends of the strands.

3. Agar plates were seeded with *Sarcina lutea* and incubated for 2 days at 25° C., so that bacterial growth was obvious. Lichen

[1] From the Field Studies Section, Tuberculosis Control Division. This work was carried out at the Hopkins Marine Station and the Rockefeller Institute for Medical Research.

strands were then placed on the agar surface and the plates again incubated. Clear areas, which expanded as incubation time increased, appeared about the strands.

4. The lichen was spread over a layer of wet Norite A, exposed to north light for 3 or 4 days, and moistened with a fine spray each day. The Norite was then eluted with acetone-alcohol and the eluate, after removal of the acetone and alcohol, was found to have antibacterial activity against *Sarcina* and against several strains of soil mycobacteria. On fractionating the eluate, the antibacterial activity was found in the fraction soluble in petroleum ether. Other fractions which appeared to have activity lost it when neutralized.

HISTORICAL BACKGROUND

A great variety of compounds, many of them crystalline, have been isolated from lichens (*2*, *3*). Zopf has described "ramalinsaure" ($C_{18}H_{14}O_9$) isolated from *Ramalina farinacea* (L) Ach. and "ramalsaure" ($C_{17}H_{16}O_7$) from *Ramalina pollinaris*. Koller and Krakauer (*4*) determined the structural formula of "cetrarsaure" ($C_{20}H_{18}O_9$), previously isolated in crystalline form by Zopf from *Cetraria islandica* (L) and *Cladina rangeferina* (L), and found it to be a xanthydrol. Diploicin was also isolated by Zopf from *Buellia canescens* (Dicks). Its composition ($C_{16}H_{11}O_5Cl_3$) and structure were determined by Nolan and his co-workers (*5*, *6*, *7*) who found it to be a diphenyl ether. They also found gangaleoidin ($C_{18}H_{14}O_7Cl_2$) obtained from *Leconora gangaleoides* to have a similar structure. Barry (*8*) found diploicin to be active against *Mycobacterium tuberculosis* and *Corynebacterium diphtherae mitis* in dilutions as low as 1:100,000. He attributed the activity to the halogenated phenyl ether structure of this compound and drew analogies with thyroxin and other phenyl ethers. Hogeboom and Craig (*9*) isolated two crystalline compounds from *Aspergillus ustus*, $C_{21}H_{17}O_6Cl_3$ (m. p. 185–187° C.) and $C_{21}H_{18}Cl_2O_6$ (m. p. 214–216° C.), which inhibited growth of *Mycobacterium ranae* at dilutions of 1:300,000 and 1:100,000, respectively. They found a second isolate, to which they attributed the formula $C_{21}H_{18}Cl_2O_6$ (m. p. 214–216° C.). Doering and coworkers (*10*) isolated three chlorine-containing compounds from the same source, one of which they called ustin and considered identical with the substance (m. p. 185–187° C.) of Hogeboom and Craig, but they assigned to it the formula $C_{19}H_{15}O_5Cl_3$. Because of the rarity of chlorinated compounds from biological sources, it is interesting to note the similarity in composition of products isolated from such apparently different sources as the lichens *Buellia* and *Lecanora* and the fungus *Aspergillus*. Since the fungal components of the lichens belong to the same family as *Aspergillus*, i. e., Ascomycetes, the similarity may be more than a

coincidence. Burkholder and Evans (*11*) tested a hundred species of lichen against *Bacillus subtilis* and *Sarcina aurea*, by placing the plants in Oxford cups, and found that 52 species inhibited growth of one of these bacteria. They found that gram-negative bacteria were generally not inhibited. Weld (*12*) obtained an antibacterial extract from the eastern Spanish moss *Tillandsia usneoides* (*Dendropogon usneoides* (*1*) Raf.)[2]

METHODS OF EXTRACTION

Method 1.—The lichen was extracted by boiling for 4 hours with acetone (2 parts) and alcohol (1 part). The extract was filtered and, after standing at room temperature with slow evaporation for a week, a copious green precipitate appeared which was filtered off.[3] The precipitate was dissolved in boiling acetone and filtered while hot. On cooling, yellow needlelike crystals appeared. The green mother liquor was decanted and the crystals washed with alcohol and acetone. They were again dissolved (the solution was now yellow), recrystallized, and washed. This process was repeated three times. With slow crystallization, crystals as long as one inch could be obtained.

Method 2.—Preliminary trial showed that with larger volumes of acetone or acetone-alcohol, cold extraction gave good yields. The cleaned lichen was packed in 6-gallon earthenware crocks and covered with acetone-alcohol (approximately 10 lb. of lichen to 60 lb. of acetone-alcohol). After standing overnight, the yellow-green solution was decanted, filtered, and poured into enamelware pans. The pans were put outdoors, protected against direct sunlight. In a brisk breeze evaporation proceeded rapidly, and in a few hours a copious green precipitate appeared and was filtered off. The red-brown mother liquor was evaporated further, until only an amorphous tan precipitate was produced. The green precipitate was dissolved in boiling acetone and filtered rapidly while hot. The filtrate was then concentrated by boiling to about one-tenth its original volume. On cooling, crystallization occurred rapidly. The green mother liquor was then decanted, and the yellow crystals were washed with cold acetone. These were then recrystallized three times, as previously described.

Method 3.—Extraction with cold acetone was carried out as described in the above paragraph. The mother liquor in this case was

[2] The eastern and the California Spanish moss are not in any way related. The former is a seed plant of the family Bromeliaceae and the latter is a lichen.

[3] The filtrate was evaporated to dryness and extracted with petroleum ether. The ether-soluble fraction (a brownish yellow noncrystalline substance) was disso ved in olive oil and tested against soil mycobacteria by the Oxford cup method and found to have strong antibacterial activity. The water-soluble fraction had ittle activity. These fractions have not been followed further.

yellow. The separation of crystals from amorphous material was carried out in the same way.

The yield by methods 2 and 3 was approximately 8 gm. of purified crystalline material per 10 lb. of lichen.[4]

PROPERTIES OF THE CRYSTALLINE MATERIAL

Solubility.—Readily soluble in hot acetone, ethyl alcohol, propylene glycol, ethyl ether. Poorly soluble in hot petroleum ether, cold alcohol, propylene glycol. Moderately soluble in cold acetone. Insoluble in water and in HCl.

Melting point.—191–192° C. when heated at an increment of 0.2° per minute, after first being brought rapidly to 160° C.

193–194° C. when heated at a uniform increment of 0.5–1.0° per minute.

The melt is brown. The crystals obtained when the melt cools are yellow-brown. The crystals melt readily in camphor. However, when the mixture is again heated, there appears to be progressive decomposition with no definite melting point.

Titration.—The substance is acid and has a neutralization equivalent of 298–310, as measured by titration in acetone.

Composition.—On analysis, no ash, nitrogen, or halide was found. The percentage composition of the batch obtained with hot acetone-alcohol extraction was (*a*) C—62.75, H—4.63, (*b*) C—62.75, H—4.69. Analysis of the batch extracted with cold acetone-alcohol gave a percentage composition of (*a*) C—63.05, H—4.49, (*b*) C—63.00, H—4.64.

The substance was found to contain no methoxyl groups. To prepare the methoxyl derivative, the substance was dissolved in acetone, diazomethane in ether was added, and the volatile material was evaporated. The ester could not be crystallized. It was distilled in a molecular still, under a pressure of 0.001 mm. Hg or less at a temperature of 140–170° C. The distillate was a resin with a percentage composition of C—63.75, H—5.26, OCH_3—9.50.

From the methoxyl content, a minimum molecular weight of 326 is obtained for the ester, corresponding to a weight of 312 for the acid, which is in agreement with the titration results. Since a substance with a molecular weight twice this size would not be expected to distill at the temperatures observed, the minimum weights may be considered to represent the actual molecular weight. The results thus indicate one acid group per molecule and an empirical formula $C_{16}H_{14}O_6$

[4] The extraction cannot be carried out successfully in the presence of metal. When cold extraction was attempted using metal drums, no crystals could be obtained. Instead, a copious red-brown precipitate was found.

(C—63.5%, H—4.63%, O—31.8%) which is in reasonably good agreement with the values found on analysis of the acid.[5]

ANTIBACTERIAL PROPERTIES IN VITRO

Three strains of soil mycobacteria, B.4.1, B.5.1, and B.18.1, were obtained from stock cultures maintained by Dr. C. B. van Niel; the others were isolated from soil under his supervision. M_2, M_5, and M_6 were from cultures in which the carbon source was iso-amyl alcohol. M_8 appeared in the culture in which phenol was the carbon source.

Table 1 shows the response of these organisms to the antibacterial substance.

TABLE 1.—*Effect of* Ramalina *crystals on soil mycobacteria*

Culture [1]	Type [2]	Color	Minimum concentration of crystals (expressed in γ/cc.) at which growth is inhibited at room temperature after 3 days	
			Partial inhibition	Complete inhibition
M_2	S	Yellow	0.50	5.0
M_5	S	Pink	5.0	>50.0
M_6 [3]	R	Pink	<5.0	5.0
M_8	R	Gray-White	5.0	>50.0
B.4.1	S	Orange	0.5	5.0
B.5.1	S	Pink	0.05	>50.0
B.18.1	R	Orange	0.05	5.0

[1] Medium: yeast extract (Difco), glucose, 0.1 percent Tween 80.
[2] S=smooth colony surface on agar.
R=rough colony surface on agar.
[3] Organism is sensitive to Tween 80. To protect against Tween 80, 0.3 percent serum albumin was added to culture.

Table 2 shows the response of various bacteria to the antibacterial substance. The bacilli *Pseudomonas*, *Salmonella*, and *Shigella* were insensitive.

To determine whether there was sterilization or only inhibition of growth, samples were taken from cultures Number 14 to 20 (table 2) at the greatest crystal concentration which showed no growth. In each case, 0.1 cc. was added to plain broth and to blood broth. These cultures were then incubated for 18 hours and examined. The following table shows the results observed:

Culture No.	14	15	16	17	18	19	20
Growth	−	−	−	−	+	+	+

[5] I am indebted to Dr. Adalbert Elek for the elementary analysis and to Dr. Lyman C. Craig for the preparation and analysis of the methoxyl derivative.

TABLE 2.—*Growth of various bacteria in the presence of* Ramalina *crystals*

[Density of growth indicated by numbers 0–4]

Species	Growth after 18 hours incubation			
	50 γ/cc.	5 γ/cc.	0.5 γ/cc.	.05 γ/cc.
1. *Klebsiella pneumoniae*	4	4	4	4
2. *Bacillus coli*	4	4	4	4
3. *Bacillus proteus*	4	4	4	4
4. *Pseudomonas pyocaneous*	4	4	4	4
5. *Salmonella aertryke*	4	4	4	4
6. *Salmonella typhi murium*	4	4	4	4
7. *Shigella* D–6 (Dubos)	3	4	4	4
8. *Shigella* VZ-4S (Goebbel)	3	4	4	4
9. *Shigella* Z-Weill (Goebbel)	3	4	4	4
10. *Shigella* Sonne (Goebbel)	4	4	4	4
11. *Staphylococcus aureus* 40 (Dubos)	4	4	4	4
12. *Staphylococcus aureus* 42-B (Dubos)	2	3	4	4
13. *Staphylococcus aureus* O'Hara (Dubos)	3	4	4	4
14. *Pneumococcus* type I SVI (Dubos)	0	3	4	4
15. *Pneumococcus* type II D-39 (Avery)	0	4	4	4
16. *Pneumococcus* type III A-66 (Avery)	0	1	3	3
17. *Streptococcus hemolyticus* T-36 (Lancefield)	0	4	4	4
18. *Streptococcus hemolyticus* T-32 (Lancefield)	0	1	3	3
19. *Streptococcus hemolyticus* T-28 (Lancefield)	0	2	3	4
20. *Streptococcus hemolyticus* H-69D (Lancefield)	0	4	4	4

NOTE: In species No. 14–20, inclusive, tests were made with and without defibrinated rabbit blood. In the presence of blood (approximately 2 percent), inhibition was the same as in broth. In species No. 18, clumping was observed in broth culture containing 5.0 and 0.5 γ/cc., but not at other concentrations.

Pneumococcus, *Streptococcus*, and some of the *Staphylococci* are inhibited by 50γ per cc. or less. Experiments designed to define more closely the minimal effective concentration in strains of *Pneumococcus* and *Streptococcus* showed complete sterilization of concentrations of 10–20γ per cc. in the former, while in the latter the variation between strains was much greater, i. e. from 10 to over 50γ per cc.[6] (table 2A).

TABLE 2A.—*Sterilization of cultures of* Pneumococcus *and* Streptococcus *by* Ramalina *crystals* [1]

Culture	Minimum concentration for sterilization in γ/cc.	Culture	Minimum concentration for sterilization in γ/cc.
1. *Pneumococcus* type I SVI Dubos	20	5. *Streptococcus* T-32 Lancefield	10
2. *Pneumococcus* type II D-39 Avery	20	6. *Streptococcus* T-28 Lancefield	>50
3. *Pneumococcus* type III A-66 Avery	10	7. *Streptococcus* H-69D Lancefield	>50
4. *Streptococcus* T-36 Lancefield	10		

[1] Tests were run in duplicate with the organisms grown in plain broth and in Avery's blood broth. Results were essentially the same in both cases.

Several strains of tubercle bacilli were tested, using Dubos' liquid medium containing 0.05 percent Tween 80 and 0.3 percent bovine serum albumin (*13*). The results are given in table 3. The three different isolates of strain $H_{37}RV$, which showed somewhat different colony morphology, are listed together, since they gave identical

[6] To test possible activity of the crystalline substance in vivo against *Pneumococcus*, mice were inoculated with type II *Pneumococcus* and then given a solution of the crystals in sesame oil three times daily subcutaneously. There was no significant difference in mortality between the treated animals and the controls.

TABLE 3.—*Growth of various strains of tubercle bacilli in the presence of* Ramalina *crystals*

[Density of growth indicated by numbers 0–5]

Strain	Days after inoculation	γ/cc. 50	20	10	5	0.5	0
$H_{37}RV$ [1] (3 isolates)	9	0	0	1	1	2	2
	11	0	0	2	3	3	4
	14	0	0	2	3	4	5
	16	0	0	3	4	5	5
	22	0	0	4	5	5	5
Waller	9	0	0	1	1	2	0
	11	0	1	1	1	2	0
	14	0	2	3	3	5	2
	16	0	2	3	3	5	3
	22	0	3	4	4	5	5
Jamaica	9	0	0	1	1	1	±
	11	0	0	1	1	2	1
	14	[2]	0	2	2	4	4
	16	[2]	0	3	3	5	5
	22	[2]	0	4	5	5	5
Torres	9	0	0	±	±	±	1
	11	0	0	2	2	1	2
	14	0	0	3	3	4	5
	16	0	0	3	3	5	5
	22	0	0	4	5	5	5
TA_3S	9	0	2	2	2	3	3
	11	1	3	3	3	5	5
	14	3	4	4	4	5	5
	16	4	4	4	4	5	5
	22	4	4	4	4	5	5
Kirchberg	9	1	3	3	3	4	4
	11	2	3	3	3	5	5
	14	4	4	4	4	5	5
	16	4	4	4	4	5	5
	22	4	4	4	4	5	5
Ravenel	9	0	0	0	0	0	0
	11	0	0	1	±	0	0
	14	0	0	1	±	0	0
	16	0	0	1	1	0	0
	22	0	1	3	3	0	0

[1] Tests were made on 3 separate isolates. Since the results were identical for all 3, they are listed together in this table.
[2] Mold contamination.

NOTE: All cultures were grown in Dubos' medium containing 0.3 percent bovine serum albumin. Each tube was inoculated with 10-day-old cultures previously grown in Dubos' medium to give a final dilution of the inoculum of 10^{-5}.

results. The human strains showed complete inhibition by concentrations of 1:50,000 and noticeable inhibition at concentrations as low as 1:2,000,000, with the exception of the Waller strain, which required a concentration of 1:20,000 for complete, and 1:200,000 for partial, inhibition. The bovine strain (Ravenel) also required a concentration of 1:20,000 for complete inhibition. The two avian strains were markedly more resistant, showing only partial inhibition at a concentration of 1:20,000. To determine whether the bacteria had been killed or merely arrested in growth, 0.5 cc. of the medium from each of the negative cultures of the human strains was inoculated intraperitoneally into guinea pigs, which were sacrificed and autopsied 7 weeks later. Only one animal, the one which received the $H_{37}RV$ containing 20γ/cc., showed tuberculosis. The other five animals showed no signs of disease.

Table 4 shows the effect of 0.1 percent serum added to the medium.[7] The inoculum in this experiment was 400 times as great as in the preceding experiment. Apparently no protective effect is afforded by serum in this concentration.

TABLE 4.—*Effect of serum on inhibition of growth of human tubercle bacilli, $H_{37}RV$, by* Ramalina *crystals*

[Density of growth indicated by numbers 0–5]

Number of days after inoculation	Dubos' medium				Dubos' medium plus 0.1 percent serum								Dubos' medium plus
	No serum				Human serum				Bovine serum				0.1 percent albumin
	Concentration of crystals, in γ/cc.												
	40	4	0.4	0	40	4	0.4	0	40	4	0.4	0	0
1	0	1	2	2	0	1	2	2	0	1	2	2	2
2	0	2	3	3	0	2	2	3	0	2	3	3	3
5	0	3	4	4	0	3	4	4	0	3	4	4	4
7	0	3	4	4	0	3	4	4	0	3	4	4	4
12	0	3	4	5	0	3	4	5	0	3	4	5	5

NOTE: Inoculum from 7-day-old culture, to give final dilution of 4×10^{-3}.
Albumin: Armour bovine serum albumin (fraction V).
All tubes were run in duplicate.
Dispersion:
4 γ crystals per cubic centimeter resulted in growth as coarse clumps.
0.4 γ crystals per cubic centimeter resulted in growth as medium clumps.
Controls with no crystals produced fine suspensions with no macroscopic clumping.

PROPERTIES IN VIVO

I. Toxicity—Crystals dissolved in sesame oil. All injections subcutaneous.

A. Mice (25 gm.):

1. *Single injections.*—2.0 mg. was lethal, death occurring within 18 hours. 1.5 mg. was not lethal. Animals survived indefinitely.

2. *Successive injections.*—An initial injection of 1.25 mg. in 0.25 cc. of oil was followed in 22 hours by a second dose and 6 hours later by a third dose. The animal showed no symptoms and was sacrificed 24 hours after the last injection. There was oil at the site of injection, but no local tissue reaction.

Animals receiving two injections of 1.25 mg. each, in 0.25 cc. of oil at 24-hour intervals, were sacrificed 7 days later. Oil was found walled off in a thin connective-tissue membrane about which was a thin pad of fatty tissue suggesting the laying down of new fat.

B. Guinea pigs (250–350 gm.):

30 mg. (10 mg./cc.), followed by a second similar dose in 24 hours, was lethal in 5 hours.

20 mg. (10 mg./cc.), given daily for 3 days, produced no symptoms.

One animal receiving two injections of 15 mg. (10 mg./cc.), with a 6-hour interval between injections, was sacrificed 7 days later. At the site of one

[7] Preliminary trial with rabbit serum showed that it contained a factor which inhibited the growth of tubercle bacilli and it was therefore not used.

injection there was a small avascular area in the skin, but no other reaction. (In this case the tip of the needle had apparently come into the dermis.) The other site (inguinal) showed a yellow-white area, about 1 x 1 x 0.4 cm., composed of fatty tissue enclosing many oil droplets. Smears taken from both sites showed monocytes laden with oil droplets.

II. Local reaction to Tween 80 and to Tween 80-oil mixtures.

Guinea pigs (350–400 gm.): All injections were subcutaneous into the inguinal region.

Tween 80 only.—0.5 cc.—2 days later. There was no visible local reaction. Smear taken from site showed occasional leucocytes (polymorphys and monocytes, neither containing fat).

2.0 cc.—3 days later. Small amount of somewhat bloody exudate. Smear showed only erythrocytes, and these appeared to be intact. Fascia at site of injection and over surrounding abdominal muscle was thickened and yellow-white.

Tween and sesame oil, 1.0 cc. Examination 24 hours after injection.

Tween	*Oil*	
9	1	—Slightly bloody exudate with very fine, fat droplets. Fascia markedly swollen and gelatinous. Vein at site of injection much larger than contralateral.
5	5	—Clear exudate containing fat droplets. Abdominal fascia swollen and gelatinous. At site of injection, fascia dense white but not swollen or thickened. Vein at site much larger than contralateral.
1	9	—No exudate, no oil. Vein at site larger than contralateral.
1	9	—No trace of oil or Tween. Vein larger than contralateral.
2	8	—No trace of oil or Tween. Vein larger than contralateral. Fasciae seem softer than normal when manipulated with forceps.
3	7	—As above.
4	6	—No oil or Tween visible. Slight gelatinous swelling of collagen confined to site of injection. Venous system more prominent and veins more dilated than contralateral.

Tween-oil-saline emulsion: 20 percent Tween in 0.9 percent saline—1 cc. injection: Examination 18 hours after injection.

20% Tween	*Oil*	
1	1	—Area in abdominal muscle (1 x 3 cm.) over site of injection bright red (appears to be hemorrhage produced by needle). Fat pad also red. No exudate.
1	9	—Fat pad slightly pinkish. Vein enlarged. No exudate, some free oil.

III. Mobilization of crystals from site of injection.

Crystals in suspension in saline, plus Tween 80.

A. Guinea pigs (350–400 gn.). Dose, 0.5 cc.

Crystals, 20 mg.[8]*; 0.1 cc. 20-percent Tween 80; 0.9 cc. saline:*

(*a*) 1 day.—Vein and venules enlarged. Fat pad seemed somewhat larger and slightly pinker than contralateral pad. Yellow mass of crystals, 3 x 5 mm., adjacent to fat pad and vein. No inflammation or exudate.

[8] 10 mg./cc. in sesame oil was completely soluble at 37° C. 20 mg./cc. in sesame oil was completely soluble at 42° C.; precipitated at 36–37° C.

(*b*) 3 days.—Vein slightly enlarged. Fat pad same as contralateral pad. Yellow mass of crystals, 3 x 5 mm. No local reaction.

(*c*) 6 days.—Vein normal. Fat pad same size as contralateral pad, but pinkish. Yellow mass of crystals, 3 x 5 mm. No reaction in tissue surrounding mass other than a slight pinkish color to the fat mass.

It was clear from the results obtained that saline suspensions did not provide an adequate means for dispersing the antibacterial agent in the animal body. Solutions in oil alone were also unsuitable, since a good deal of the oil remained in situ, although some oil may have been incorporated into the fat cells. The results obtained with aqueous solutions of Tween 80 indicated that they reduced capillary permeability locally. By adding Tween 80 to sesame oil in suitable proportions, it was possible to have the oil taken up into the circulatory system with no obvious local or systemic injury. Experiments were therefore performed to determine whether it would be possible to have an adequate amount of the antibiotic taken into the circulation along with the oil.

The following shows the results obtained with mice:

B. Mice (25 gm.)

Solution, 5 mg. crystals in 1 cc. of oil plus Tween 80, in the proportion of 5 mg. crystals, 0.1 cc. Tween 80, 0.9 cc. sesame oil.

Dose		
cc. solution	*mg. crystals*	*Local reaction after 24 hours*
0.4	2	soft white fatty tissue, few oil droplets.
0.2	1	fatty tissue with slight fibrosis, no oil or exudate.
0.1	0.5	slight increase in fatty tissue, no fibrosis, oil or exudate.
0.05	0.25	very slight increase in fatty tissue, no other change.

One-half to one mg. in 0.1–0.2 cc. oil containing 10 percent Tween 80 could be taken up without appreciable local damage.

By trial it was found that 0.1 cc. of a 20-percent solution of Tween 80 in saline added to 0.9 cc. oil produced a fine stable emulsion. The crystals were then dissolved in oil and the solution made into an emulsion, by the method just given. When emulsified, some of the crystalline material precipitated out, but was easily resuspended. The following results show that 20 mg. given in a 1-cc. emulsion was still not completely absorbed after 4 days, whereas 10 mg. in the same volume of emulsion was completely absorbed in 2 days.

C. Mobilization of crystals from site of injection in guinea pigs.

Crystals in saline-Tween-80 emulsion.

Guinea pigs (250–320 gm.). Dose, 0.5 cc. subcutaneously, inguinal.

1. Crystals, 20 mg.; 0.1 cc. 20-percent Tween 80 in saline; 0.9 cc. oil:

(*a*) 1 day.—Vein and venules enlarged, fat pad pinkish, yellow mass of crystals, 3–4 mm.

(*b*) 2 days.—Vein enlarged, fat pad pinkish and larger than in (a). Yellow mass of crystals much smaller than in (a), 1–2 mm. in diameter.

(*c*) 4 days.—Vein slightly enlarged, fat pad slightly hemorrhagic, yellow mass 1 x 2 mm.

2. Crystals, 10 mg.; 0.1 cc. 20-percent Tween 80 in saline; 0.9 cc. oil; 0.5 cc. subcutaneous, inguinal:

2 days.—Fat pad enlarged; pinkish, with slight fibrosis. No crystals. A few free fat droplets visible in fat pad. Vein and venules dilated. Skin directly in contact with injected mass avascular, 3 mm. in diameter. No inflammation or necrosis in or surrounding this area.

Since it appeared feasible to administer the antibacterial substance in adequate amounts, an exploratory experiment on the effect of this material on tuberculosis in guinea pigs was undertaken. Thirty virgin female guinea pigs were distributed into four groups so that each group had the same weight distribution, the range in weight being 330 to 420 gm. A fifth group of four animals in approximately the same weight range was inoculated with tubercle bacilli, but was not otherwise treated. Animals in groups I, III and V (see table 5)

TABLE 5.—*Effect of* Ramalina *crystals on weight of normal and tuberculous guinea pigs*

Group	Treatment	Animal number	Weight in grams			Change in weight in grams		Percentage change in weight	
			Initial	14th day	29th day	0–14 days	14–29 days	0–14 days	14–29 days
I	Tubercle bacilli. Crystals in Tween-80-oil	517	331	321	(288) (18 days)	−10	(−33) (18 days)	−3.0	−10.3
		3586	350	331	305	−19	−26	−5.4	−7.8
		3587	356	353	338	−3	−15	−0.8	−4.3
		2417	375	372	325	−3	−47	−0.8	−12.6
		3591	378	371	310	−7	−61	−1.9	−16.4
		3589	383	368	345	−15	−23	−3.9	−6.2
		3581	387	381	285	−6	−96	−1.6	−25.2
		3593	392	410	421	+18	+11	+4.6	+2.7
		3582	396	382	329	−14	−53	−3.5	−13.9
		3583	400	425	449	+25	+24	+6.3	+5.6
III	Tubercle bacilli. Tween-80-oil	2633	342	353	270	+11	−72	+3.2	−23.5
		3584	351	336	(269) (25 days)	−15	(−82) (25 days)	−4.3	−19.9
		3560	360	361	287	+1	−73	0	−20.5
		3568	378	362	269	−16	−109	−4.2	−25.7
		3562	383	355	(276) (24 days)	−28	(−107) (23 days)	−7.3	−22.2
		3564	385	390	308	+5	−77	+1.3	−21.0
		3557	389	406	370	+17	−19	+4.4	−8.9
		3597	394	418	282	+24	−112	+6.1	−32.5
		3576	399	465	477	−34	+78	−8.5	+2.6
		3590	403	412	327	+9	−76	+2.2	−20.6
II	No tubercle bacilli. Crystals in Tween-80-oil	3580	343	380	393	+37	+13	+10.8	+3.4
		3596	368	396	432	+28	+36	+7.6	+9.1
		3569	385	385	415	0	+30	0	+7.8
		3588	390	416	456	+26	+40	+6.7	+9.6
		3573	411	440	480	+29	+40	+7.1	+9.1
IV	No tubercle bacilli. Tween-80-oil	3578	345	368	379	+23	+11	+6.7	+3.0
		3548	375	419	449	+44	+30	+11.7	+7.2
		3598	386	413	445	+27	+32	+7.0	+7.7
		3592	392	444	481	+52	+37	+13.2	+8.3
		3556	412	455	518	+43	+63	+10.2	+13.9
V	Tubercle bacilli only	3547	327	326	312	−1	−14	0	−4.3
		3575	423	436	386	+13	−50	+3.0	−11.3
		3561	323	315	(259) (28 days)	−8	−56	−2.5	−17.8
		3551	413	391	301	−22	−91	−5.2	−23.3

were inoculated intraperitoneally with 0.01 mg. of tubercle bacilli suspended in a saline-oil emulsion containing 0.05 percent Tween 80. The bacteria were obtained from a 16-day-old culture grown in Dubos' medium. Groups I and II received injections of the antibacterial substance; groups III and IV received injections of the solvents only, in the same amounts and according to the same schedule used for groups I and II. Group V was untreated. Thus, groups II and IV were nondiseased animals acting as controls for groups I and III, while group V was the control for the possible effect of the solvents on the course of the disease.

The schedule for injections is given below. All injections were made into the inguinal region and successive injections were alternated from right to left side.

Dose schedule[1]

Date	Crystals (in milligrams per day)	Sesame oil (in cubic centimeters per day)	Tween 80 (in cubic centimeters per day)	Type of mixture
Aug. 5–Aug. 8	20	2	0.4	Suspension.
Aug. 9–Aug. 10	20	1	0.1	Suspension.
Aug. 11–Aug. 18	10	0.5	0.01	Emulsion.
Aug. 19–Aug. 25	10	1.0	0.02	Emulsion.

[1] If a small amount of the antibacterial agent dissolved in oil containing Tween 80 is injected intradermally, there is immediate blanching, followed by necrosis and ulceration. There is no inflammation of the surrounding tissue and the ulcers heal rapidly.

During the period August 11–August 18, the suspension was administered in single daily doses. During the other periods, the dose was given in two injections, 10–12 hours apart.

By August 9, many of the animals showed a thickening of the skin at the site of injection or over the whole abdomen. By August 11, the swelling was much reduced and disappeared in the next few days.

No injections were given after the twentieth day.

Figures 1 to 5 show the change in weight of the animals in the course of the experiment. Animals which were not inoculated with tubercle bacilli showed a gain in weight after the first week. The untreated tuberculous animals showed little or no weight gain and began to lose weight rapidly 2 weeks after inoculation. Nine of the tuberculous animals which received only the oil injections showed little change in weight during the first 2 weeks, after which there was a rapid and continuous loss in weight. The tenth animal continued to gain weight up to the twenty-seventh day. In group I (tuberculous animals treated with crystals in oil), as in group III, there was little change in the first 2 weeks. There was then an appreciable loss of weight in all but two of the animals in the following week. These two animals then gained weight until the end of the experiment.

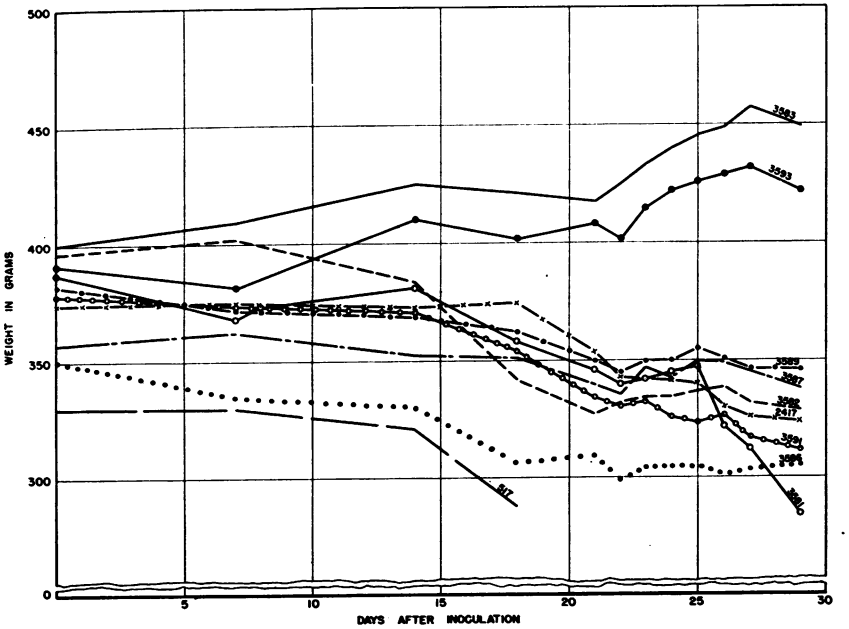

FIGURE 1.—Inoculated with tubercle bacilli, treated with crystals in oil-Tween-80. Group I

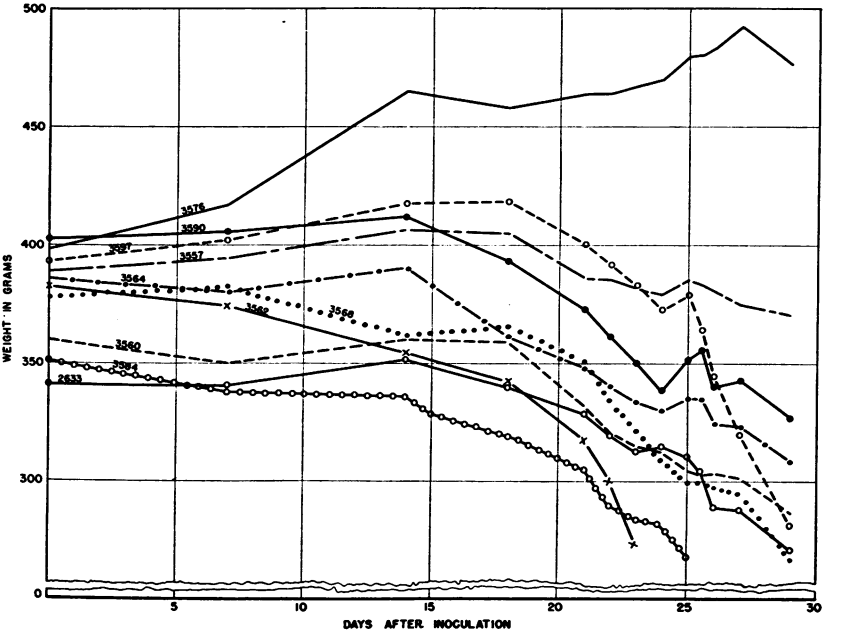

FIGURE 2.—Not inoculated with tubercle bacilli, treated with crystals in oil-Tween-80. Group III.

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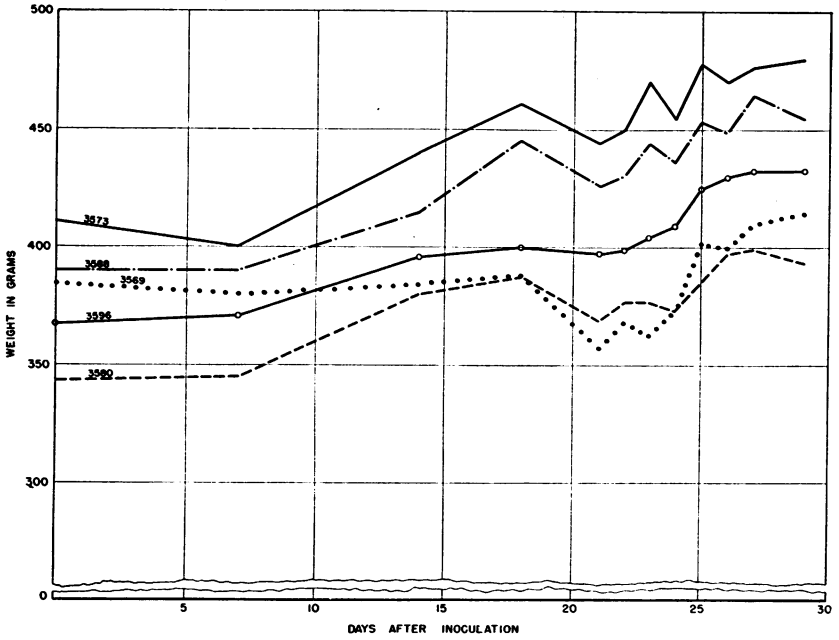

FIGURE 3.—Not inoculated with tubercle bacilli, treated with crystals in oil-Tween-80. Group II.

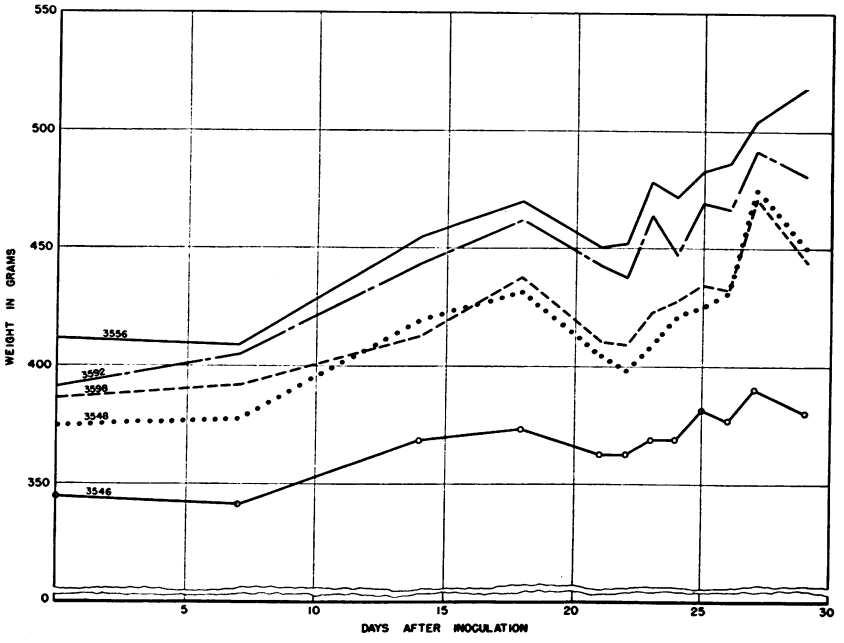

FIGURE 4.—Inoculated with tubercle bacilli, treated with oil-Tween-80 only. Group IV.

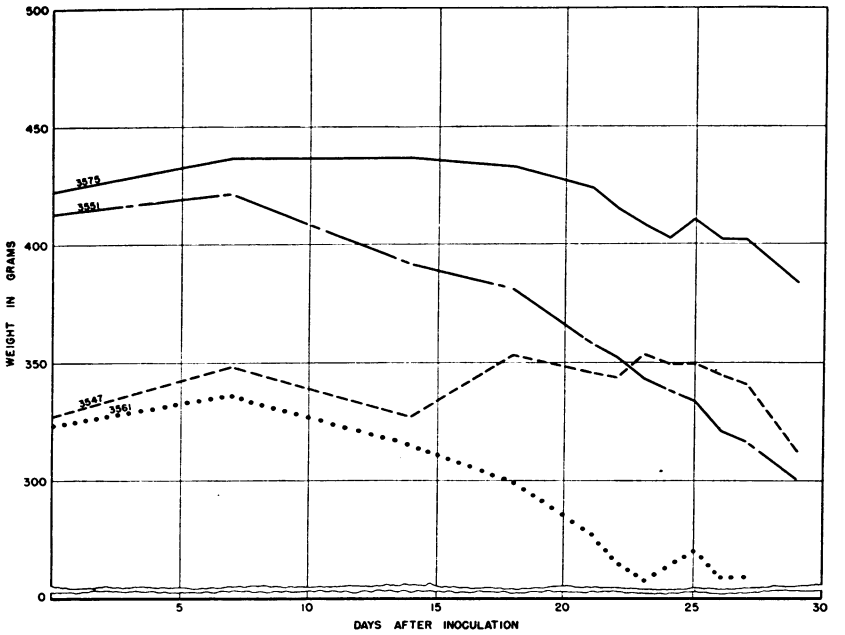

FIGURE 5.—Inoculated with tubercle bacilli, no treatment. Group V.

One animal (in the lowest weight group) died on the eighteenth day and was found on autopsy to have severe tuberculosis. Another animal, No. 3581, showed a precipitous loss in weight, beginning on the twenty-sixth day. It died 3 days later, but on autopsy showed very little tuberculosis. The weights of the other animals in this group remained at about the same level until the end of the experiment. The change in weight for each animal is shown in tabular form in table 5. In group III, there was a weight loss of 20 percent or more in each surviving animal, with the exception of two animals. In group I, animal No. 3581 showed a weight loss of 25 percent. With the exception of this animal, the loss in weight for animals in group I was appreciably and consistently lower than for animals in group III.

Since the distribution of weights at the start of the experiment was the same in both group I and group III, a simple comparison may be made of the total weight loss in each group. There were nine animals in group I, and eight in group III which survived 29 days. In group I, the weight of these surviving animals decreased from 3,388 grams on the fourteenth day to 3,107 grams on the twenty-ninth day, a decrease of 8 percent. In group III, the weight change during this period was from 3,167 grams to 2,590 grams, representing a weight loss of 19 percent. In other words, during the last 2 weeks, the surviving animals in group III lost more than twice as much weight as those in group I.

Injections were discontinued after the twentieth day and no further treatment given until the experiment was terminated on the thirty-second day, in order to allow disease to develop which might have been arrested but not eradicated during the first 20 days. All surviving animals were then sacrificed and autopsied. The extent of involvement of lung, liver, spleen, lymph nodes and omentum was estimated as "severe," "medium," "very slight," and "none"; in the lung, by the amount of consolidation; in the liver, by the number and size of "tubercles"; in spleen and lymph nodes, by enlargement; and in the omentum, by fibrosis. A rough estimate of the severity of the disease could be made on this basis (table 6).

In group I, there were two animals which could be classified as

TABLE 6.—*Findings at autopsy*

Animal No.	Days after inoculation	Died or sacrificed	Percentage change in weight	Lesions [1]					Estimated severity of disease
				Lungs	Liver	Spleen	Nodes	Omentum	
GROUP I. CRYSTALS, TWEEN 80 AND OIL									
517	18	d	−13	m	s	s	o	2	s
3581	31	d	−26	m	o	o	o	5	o–v
3586	32	s	−13	m	m	o	o	3	m
3587	32	s	−2	m	m	s	v	6	m
2417	32	s	−13	m	s	m	m	4	m
3591	32	s	−18	m	v	v	o	6	v
3589	32	s	−10	v	v (scars)	o	o	5	v
3593	32	s	+7	o	o (scars)	m	o	1	o–v
3582	32	s	−17	s	s	m	m	5	s [2]
3583	32	s	+12	o	o	o	o	2	o
Total									2 s, 3 m, 5 o–v
GROUP III. TWEEN 80 AND OIL									
3562	24	d	−28	s	s	s	m	2	s
3584	25	d	−23	m	s	s	m	3	s
3568	30	d	−29	s	s	s	m	5	s
3597	30	d	−29	s	s	s	m	4	s
2633	32	s	−21	s	s	s	s	4	s
3560	32	s	−20	s	m [3]	s	s	4	s
3564	32	s	−20	s	s	s	s	1	s
3557	32	s	−5	s	m	s	m	6	s
3576	32	s	+20	v	m	s	s	6	m
3590	32	s	−19	s	s	s	s	3	s
Total									9 s, 1 m
GROUP V. NO TREATMENT									
3561	28	d	−20	m	s	s	s	4	s
3547	31	d	−5	s	s	s	s	5	s
3551	32	d	−27	m	s	s	s	5	s
3575	32	s	−9	s	s	s	m	2	s
Total									4 s

[1] Symbols:
o, no lesions
v, very slight
m, mild
s, severe
1–6, extent of fibrosis of omentum

[2] Entire right flank filled with liquid odoriferous pus. Abdomen much swollen.

[3] Many tubercles on diaphragm and lining of peritoneal cavity.

having severe disease. One of these, No. 517, died in the early stages of the experiment; the other, No. 3582, was found to have a huge infected abscess containing nonacid-fast gram-negative bacilli, which spread from the groin across the entire flank and abdomen. Three animals had mild, and five had very slight or no disease. In group III, one animal had mild disease, while in the other nine, disease was severe.

Mortality is shown in table 7. There were twice as many deaths in the controls as there were in the treated group. Thus, on the basis of weight change, mortality, and findings at autopsy, the group of animals treated with the crystalline substance showed much less disease than the controls.

TABLE 7.—*Guinea pig tuberculosis mortality*

Group I			Group III			Group V		
Animal No.	Days after inoculation	Weight loss, in grams	Animal No.	Days after inoculation	Weight loss, in grams	Animal No.	Days after inoculation	Weight loss, in grams
517	18	43	3562	24	107	3561	28	64
3581	31	120	3584	25	82	3547	31	24
			3568	30	109	3551	32	131
			3597	30	102			
Fraction dead, 0–32 days	2/10		Fraction dead, 0–32 days	4/10		Fraction dead, 0–32 days	3/4	

CONCLUSION

A crystalline substance has been isolated from the lichen *Ramalina reticulata*, with a melting point of 191–192° C. and an empirical formula of $C_{16}H_{14}O_6$. It can be administered subcutaneously in oil, daily, at a rate of 10–20 mg. per 350–400 gm. guinea pig for a period of 3 weeks without obvious toxic effects. When so administered to guinea pigs infected intraperitoneally with human tubercle bacilli of the strain $H_{37}RV$, it appears to retard the progress of the disease.

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DISSEMINATED PULMONARY CALCIFICATION [1]

A Report of 113 Cases

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During the past 30 years, there has been considerable discussion and speculation in American medical literature concerning the possible cause of disseminated pulmonary calcification. It has been suggested that such calcification represents healed miliary tuberculosis (*1*, *2*, *3*, *4*, *5*) or healed tuberculous bronchopneumonia (*5*). Sutherland (*6*) suspected that abnormalities in calcium metabolism were responsible. Sayers and Meriwether (*7*) found 125 instances of disseminated pulmonary calcification among approximately 18,000 miners in Picher, Okla., and suggested that in addition to healed miliary tuberculosis, a pneumomycosis should be considered as a possible etiologic agent. Geever (*8*) noted two instances of such calcification in nonreactors to tuberculin, but was unable to determine the etiology from his roentgenographic and histopathologic material. Lumsden and Dearing (*9*) found 11 cases which they considered to be of miliary calcification, in a survey conducted in Giles County, Tenn. Ten of these cases were found among 4,377 whites, and one among 983 Negroes, a rate of 2.3 per thousand and 1.0 per thousand, respectively. Long and Stearns (*10*) pointed out that pulmonary calcifications were observed in over 15 percent of the draft inductees from those stations "bounded roughly by Fort Oglethorpe, Ga.; Jefferson Barracks, Mo.; Little Rock, Ark.; and Columbus, Ohio." "Also disseminated 'miliary' calcifications . . . seemed relatively more frequent in this area." From films taken in preemployment examinations in various Indiana industries, Spolyar (*11*) collected approximately 65 cases which he regarded as possible instances of healed pulmonary aspergillosis.

It should be noted that most cases of disseminated pulmonary calcification have been found in the central region of the United States. It has been observed for more than 20 years that many people in this region have pulmonary calcification but do not react to tuberculin. Palmer (*12*) has noted that in this area nontuberculous pulmonary calcification is most frequently found in persons who react to histoplasmin. He has also pointed out (*13*) that in the United States significant geographic differences exist in the levels of histoplasmin sensitivity and, furthermore, that these levels are highest in the central region. Zwerling and Palmer (*14*) reported 15 persons who showed disseminated pulmonary calcification, and noted that 14 reacted to histoplasmin.

[1] From the Field Studies Section, Tuberculosis Control Division.

The cases of disseminated pulmonary calcification to be presented in this paper have been restricted to those instances in which at least five separate calcareous deposits were noted in each lung field. Further, the deposits must have been scattered over at least one-half of each lung field. In almost every instance, these minimal requirements were exceeded. Through adherence to these criteria, disseminated pulmonary calcifications may be divided into two groups. The first is designated "miliary calcification" (fig. 1). The calcifications are small, round, uniform in size, numerous, and widely and symmetrically scattered throughout each lung field. This type is sometimes called "wheatena" or "buckshot" calcification. The second group is designated "multiple bilateral calcification" (fig. 2). In these instances the calcareous deposits are fewer in number, often irregular in outline, of varying size, and often distributed in an asymmetric pattern. In each group, two subgroups may be made, according to whether calcareous deposits are observed in the hilar regions.

The distinction between the two groups is of interest because most observers feel that the "miliary" type results from hematogenous dissemination of the causative agent (*1*, *2*, *3*, *4*, *5*), whereas bronchogenic dissemination produces the "multiple bilateral" type (*5*).

MATERIAL AND METHODS

From various sources, 113 instances of disseminated pulmonary calcification were collected. Sixty-four of these were observed among a group of school children in Kansas City, Mo., for which considerable data have been reported by Furcolow, High, and Allen (*15*). It appears appropriate to consider these 64 cases separately so that they may be compared with the Furcolow, High, Allen report. The remaining 49 cases will also be discussed here.

All of the roentgenograms were read by two men, each experienced in the interpretation of pulmonary calcification. The 113 cases of disseminated calcification, found by either reader, were reviewed by both, first separately, and then together. The classification of these cases represents the final opinion of the two readers. The intradermal tuberculin and histoplasmin tests were given and read by two small groups that have worked together for several years. Each group used similar antigens and similar criteria for interpretation of the tests. The tuberculin used was 0.0001 mg. of PPD-S, furnished by Dr. Florence Seibert of the Henry Phipps Institute, University of Pennsylvania, Philadelphia; the histoplasmin, furnished by Dr. C. W. Emmons of the National Institute of Health (*16*), was a 1 to 1,000 dilution of his lot H_3. A reaction to both tuberculin and histoplasmin was considered positive if the induration measured 5 or more millimeters in diameter at the 48-hour reading.

FINDINGS

A study was recently conducted in Kansas City, Mo., by the Tuberculosis Control Division of the United States Public Health Service, with the cooperation of the City Health Department, Board of Education, and Tuberculosis Society, to determine various epidemiologic factors related to histoplasmin sensitivity. Approximately 16,000 children of school and preschool age were given intradermal tuberculin and histoplasmin tests, and were examined with an 11″ x 14″ or 14″ x 17″ roentgenogram of the chest.

Among this group of 15,980 children, whose ages ranged from less than 1 to 18 years, 64 instances of disseminated pulmonary calcification were found. The distribution of these cases according to age and sex for the white children is presented in table 1. Similar

TABLE 1.—*Cases of disseminated pulmonary calcification per 1,000 persons, by age, race, and sex. School children, Kansas City, Mo., 1945*

Age [1] (years)	White and Negro			White								
	Both sexes			Male			Female			Both sexes		
	Number		Rate per 1,000 persons tested	Number		Rate per 1,000 persons tested	Number		Rate per 1,000 persons tested	Number		Rate per 1,000 persons tested
	Children	Cases		Children	Cases		Children	Cases		Children	Cases	
0–4	242	0	0	119	0	0	88	0	0	207	0	0
4–6	2,482	2	.8	1,083	2	1.8	1,001	0	0	2,084	2	1.0
7–9	3,594	9	2.5	1,415	2	1.4	1,442	5	3.5	2,857	7	2.5
10–12	3,628	14	3.9	1,417	7	4.9	1,451	7	4.8	2,868	14	4.9
13–15	3,966	21	5.3	1,792	9	5.0	1,887	11	5.8	3,679	20	5.4
16–18	2,068	18	8.7	837	7	8.4	965	11	11.4	1,802	18	10.0
Total	15,980	64	4.0	6,663	27	4.1	6,834	34	5.0	13,497	61	4.5

[1] Age last birthday.

data are not presented for Negro children, since too few instances of such calcification were observed among them. It will be noted that among whites, the frequency of this type of pulmonary calcification rises steadily in successively older age groups. No cases were found among the 207 white children under 4 years of age, but in the age group 4–6, a frequency of 1.0 per thousand was found, and in the age group 16–18 this rate had risen to 10.0 per thousand. The findings are presented in figure 3.

Although the difference between white males and females is not statistically significant, it is of some interest to note that slightly more females than males presented this type of calcification.

If the rate of 4.5 per thousand found for white children were the same for Negroes, 11 cases would be expected among the 2,483 Negro

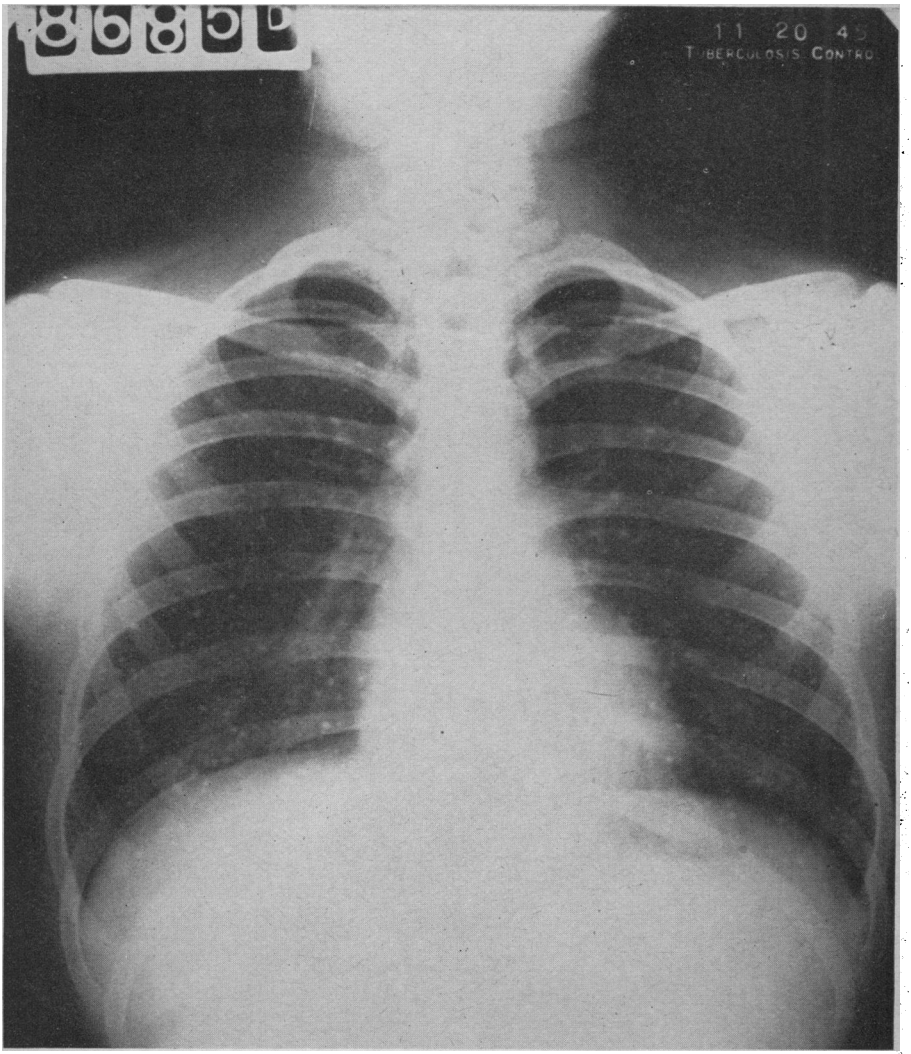

FIGURE 1.—Miliary type of pulmonary calcification (tuberculin negative, histoplasmin positive). Over 100 separate calcareous deposits are present in each lung field.

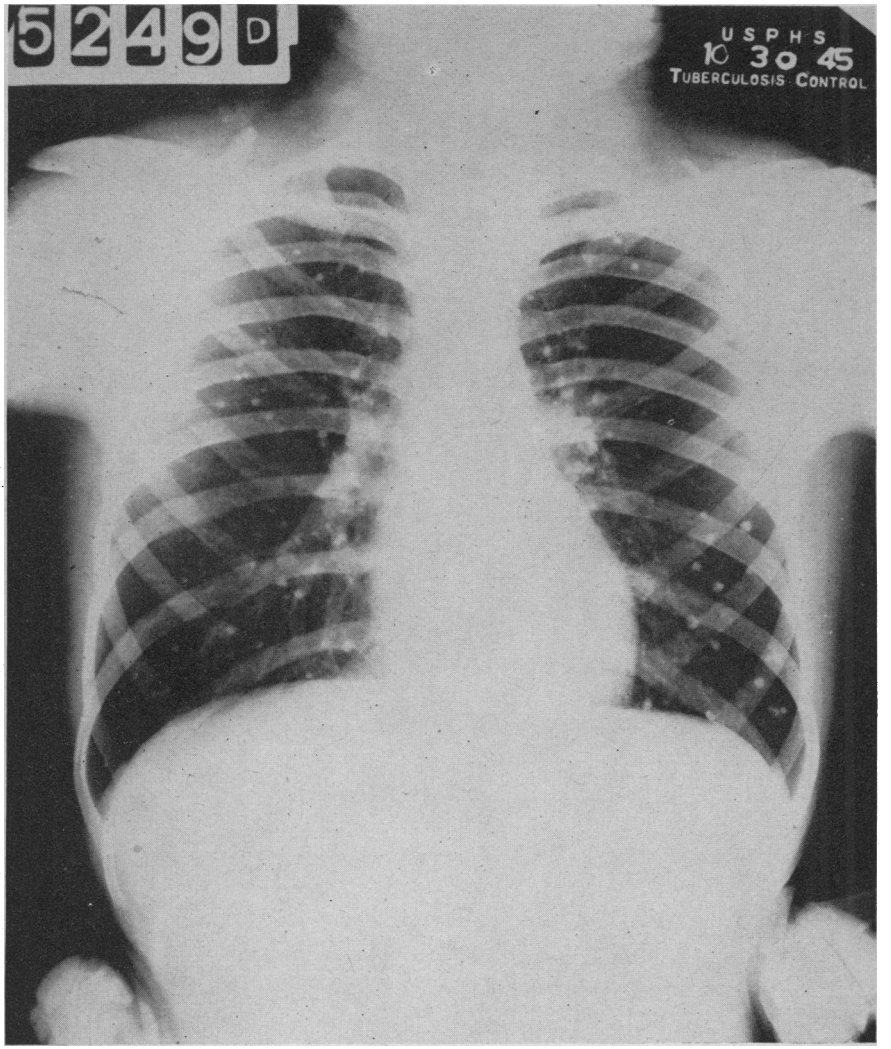

FIGURE 2.—Multiple bilateral type of pulmonary calcification (tuberculin negative, histoplasmin positive).

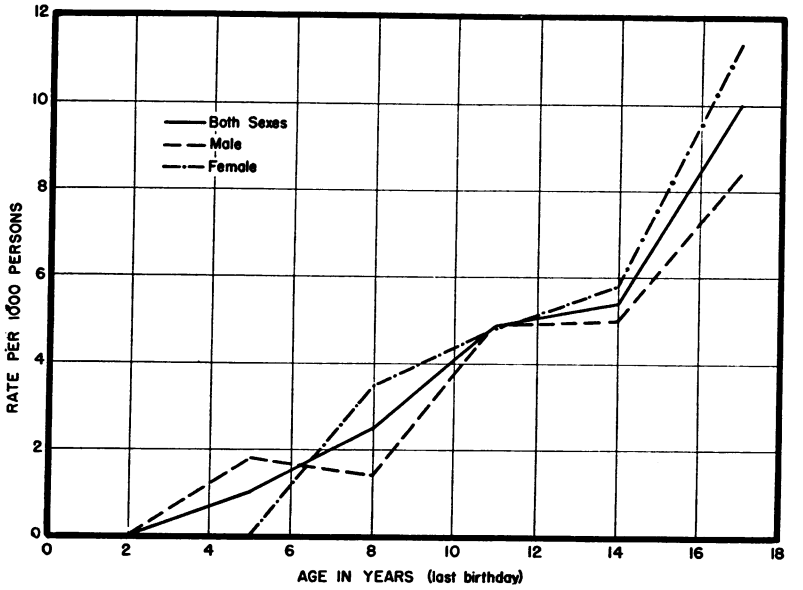

FIGURE 3.—Cases of disseminated pulmonary calcification per 1,000 persons by age and sex. Kansas City, Mo., white school children.

children. Actually, only 3 instances of disseminated pulmonary calcification were observed. Two males, one 9 and one 13 years of age, and one female 8 years of age presented this type of calcification. The observed rate for the Negro children is 1.2 per thousand, approximately one-fourth of that found for the whites. This racial difference appears to be statistically significant. Lumsden and Dearing (*9*), in the survey made in Giles County, Tenn., observed approximately the same racial difference.

Furcolow et al. (*15*) reported epidemiologic data from the same group of children. They found an increase with age in the frequency of all types of pulmonary calcification. The frequency of disseminated pulmonary calcification likewise shows an increase with age.

Of the 64 cases of disseminated calcification, 24 showed calcification in miliary patterns. Sixteen of these did not have calcareous deposits in the tracheobronchial lymph nodes, whereas the other 8 showed calcification in these structures. The remaining 40 cases presented multiple bilateral calcifications, 27 of which were associated with calcareous deposits in the tracheobronchial lymph nodes and 13 of which showed no such deposits. Of those with the miliary type, only 33.3 percent had calcareous deposits in these structures, whereas 67.5 percent with the multiple bilateral type had such deposits. Moreover, the calcareous deposits in the hilar areas in the multiple bilateral type tended to be larger and to contain more individual pieces of

calcium. These observed differences in hilar calcification may represent significant differences in the pathogenesis of these two types of disseminated calcification. The miliary type may represent hematogenous dissemination of the causative agent. The multiple bilateral type may be caused by bronchogenic spread, or by multiple "primary" foci.

In only 1 of the 64 cases of disseminated calcification was any other abnormality noted in the roentgenogram of the chest. In this 1 case, obliteration of the left costophrenic sulcus was seen. The remaining 63 cases did not show changes such as fibrosis, deviation of the trachea, localized or generalized emphysema, retraction of the lung root, atelectasis, etc.

In 62 of the 64 cases found in this study, tuberculin and histoplasmin tests were given. The results of these tests are presented in table 2. It is to be noted that 93.5 percent of those tested reacted only to histoplasmin, while none reacted to tuberculin alone. In 3.2 percent, neither skin test was positive, and in 3.2 percent, both skin tests were positive. Thus, disseminated pulmonary calcification was associated with a positive histoplasmin reaction in 96.8 percent of the cases. In the reactions to histoplasmin, there was no significant difference between the miliary and the multiple bilateral types.

It should be stated that all types of pulmonary calcification observed among these Kansas City school children were more frequently found in histoplasmin reactors than in tuberculin reactors. Furcolow et al. studied 6,528 school children who were part of the same group in which the cases of disseminated pulmonary calcification were found. They included only those children, however, whose chest roentgenograms were entirely satisfactory for interpretation of all types of calcification. The present report deals with the entire group because it is felt that disseminated calcification would be seen even on films of poor technical quality. Furcolow et al. found 828 cases of pulmonary calcification among the 6,528 school children. Of the 828 cases, 56, or 6.8 percent, occurred among children positive to both skin tests. Among those who reacted only to histoplasmin, 649 cases, or 78.4 percent, were found. Thirty-one cases, or 3.7 percent, were found among those who reacted only to tuberculin; and 92, or 11.1 percent, were found among those who reacted to neither test. In table 2, these results are compared with those found among the instances of disseminated calcification.

It is important to note that the percentage of histoplasmin reactors

was higher among those with disseminated calcification than among those with all types of pulmonary calcification. Among the former, 96.7 percent reacted to histoplasmin (or to histoplasmin and to tuberculin); and among the latter, only 85.2 percent reacted. The difference is significant.

TABLE 2.—*Percentage distribution by reactions to histoplasmin and tuberculin for all children tested—for children with pulmonary calcification and for children with disseminated calcification. School children, Kansas City, Mo., 1945*

Skin reaction	Among 6,528 school children [1]				Disseminated calcification among 15,980 school children	
	All children		Children with all types of pulmonary calcification		Total	Percentage
	Total	Percentage	Total	Percentage		
H+ T−	2,454	37.6	649	78.4	58	93.5
H+ T+	235	3.6	56	6.8	2	3.2
H− T+	273	4.2	31	3.7	0	0
H− T−	3,566	54.6	92	11.1	2	3.2
Total	6,528	100.0	828	100.0	62	100.0

[1] From Furcolow et al. (*15*).

From the above findings, it appears that tuberculosis, contrary to the opinion of many previous writers, is not the cause of the majority of such calcifications. Among the 62 cases, 60, or 96.7 percent, did not react to tuberculin, and only 2, or 3.2 percent, reacted to tuberculin as well as to histoplasmin. Less than one-third as many persons with disseminated calcification reacted to tuberculin as did persons with all types of pulmonary calcification.

From the data available for this group of children, it is impossible to state conclusively that these disseminated calcifications are caused by the agent producing histoplasmin sensitivity; but it seems more likely that they were caused by that agent than by the tubercle bacilli.

Of the 64 cases, 52 were found in as many families, while in each of 6 families, 2 siblings presented the same findings. Such unusual calcification, which occurs at a rate of 4 per thousand in the school population, is extremely unlikely to have occurred by chance in the siblings of 6 separate families. Five of the pairs were white, and one pair Negro. It should be noted incidentally that the Negro brothers were the only Negro males among the 1,155 studied who showed this type of calcification. In no case did the age difference between the

2 siblings exceed 4 years, and in 4 of the pairs the age difference was 2 years or less. In only 1 pair were children of unlike sex affected. These findings are summarized in table 3.

TABLE 3.—*Siblings showing disseminated pulmonary calcification by sex and age. School children, Kansas City, Mo., tested in 1945*

Family	Sex	Age [1]
1	F	11
	F	12
2	F	10
	F	12
3	M	9
	M	13
4	F	12
	F	14
5	M	6
	F	9
6	F	16
	F	17

[1] Age last birthday.

In another pair of siblings, incompletely calcified miliary densities were noted in one, and disseminated noncalcareous miliary densities were noted in the other. The findings in this pair suggest that the two children may have developed active disease at about the same time.

From sources other than the Kansas City survey, an additional 49 cases were found that presented this type of pulmonary calcification. Fifteen of these were previously reported by Zwerling and Palmer (*14*). Twenty-nine of the forty-nine cases were found in approximately 13,000 children and adults living in Kansas and Missouri. Nineteen cases were found among nearly 15,000 student nurses who studied in 72 training schools in 10 cities throughout the United States. One case was that of a young man whose residence was not stated. The age of the 49 ranged from 10 to 75 years. Two cases were found in siblings.

Forty-six cases were tested with tuberculin and histoplasmin, and again this type of calcification was found most frequently in histoplasmin reactors (table 4). The percentage that reacted only to his-

TABLE 4.—*Cases of disseminated pulmonary calcification discovered in sources other than the Kansas City survey, according to reaction to histoplasmin and tuberculin*

Skin reaction	Number	Percentage
H+T−	35	76.1
H+T+	9	19.6
H−T+	0	0
H−T−	[1] 2	4.3
Total	46	100.0

[1] Also doubtful reaction to histoplasmin (1 case).

toplasmin was 76.1; none reacted only to tuberculin. The percentage that reacted to both skin tests was 19.6; and 4.3 percent reacted to neither, although one had a doubtful reaction to histoplasmin. Those who showed positive reactions to histoplasmin totaled 95.7 percent.

Only 1 of the 49 cases had a lesion other than disseminated calcification, demonstrated by the roentgenogram. In this instance, obliteration of the left costophrenic sulcus was present, and there were also minimal changes suggestive of thickened pleura overlying the right apex.

When all available cases are combined, 113 instances of disseminated pulmonary calcification have been found in approximately 45,000 persons. One hundred and eight of these were tested with both tuberculin and histoplasmin. Two were tested only with tuberculin and did not react. The results of these tests are presented in table 5.

TABLE 5.—*Cases of disseminated pulmonary calcification collected from all sources, according to reaction to histoplasmin and tuberculin*

Skin reaction	Total	Multiple bilateral type		Miliary type	
		With hilar calcification	With no hilar calcification	With hilar calcification	With no hilar calcification
H+T−	93	45	15	16	17
H+T+	11	5	1	3	2
H−T+	0	0	0	0	0
H−T−	[1] 4	0	1	1	[1] 2
Not tested	5	1	1	[2] 2	[2] 1

[1] Also doubtful reaction to histoplasmin (1 case).
[2] 1 case negative to tuberculin, not tested with histoplasmin.

No case was found with a positive tuberculin reaction alone, whereas 86.1 percent were found in those who reacted to histoplasmin alone. Of the 108 cases, 3.7 percent reacted to neither skin test, and 10.2 percent reacted to both. Therefore, 96.3 percent of the cases showing this type of calcification reacted to histoplasmin, and only 10.2 percent reacted to tuberculin.

Of the total group of 113 cases, 69, or 61.1 percent, were of the multiple bilateral type; while only 44, or 38.9 percent, were of the miliary type. Calcifications were noted in the hilar structures in 73.5 percent of the former type, whereas only 50.0 percent of the latter type showed such calcifications. No significant differences were observed in the skin reactions to histoplasmin or tuberculin in these two groups, regardless of the presence or absence of calcifications in the hilar areas.

From those histories of residence that were available, it was learned

that over 75 percent of the individuals with disseminated pulmonary calcification had lived all or most of their lives in areas where Palmer found high histoplasmin reaction rates.

SUMMARY

One hundred and thirteen instances of disseminated pulmonary calcification are reported, and the skin reactions to tuberculin and histoplasmin are given.

From 64 cases of such calcification, found in a survey of 15,980 school children in Kansas City, Mo., the following observations were made:

1. The frequency among the whites rose steadily from none in the age group under-4-years to 10 per 1,000 in the age group 16–18.
2. Negroes showed less calcification of this type than whites—1.2 per 1,000 in the former and 4.5 per 1,000 in the latter.
3. A definite familial relationship was noted.
4. Only 1 of the 64 cases showed roentgenographic abnormalities other than disseminated calcification.
5. In no instance was such calcification noted among those who reacted only to tuberculin; but in 58 instances, or 93.5 percent of the group, disseminated calcification was noted among reactors to histoplasmin alone. In two instances, or 3.2 percent, the children reacted to tuberculin and histoplasmin, and in two other instances, to neither antigen. Of this group, 96.7 percent reacted to histoplasmin.

From other sources, 49 additional instances of disseminated calcification were found. Of these, 76.1 percent reacted only to histoplasmin, and none only to tuberculin. The percentage of cases that reacted to both antigens was 19.6, and 4.3 percent reacted to neither antigen. The percentage of cases that reacted to histoplasmin was 95.7.

Of the 113 cases, 108 received tests with tuberculin and histoplasmin. One hundred and four cases, or 96.3 percent, reacted to histoplasmin, while only 4 had negative reactions to this antigen. None reacted only to tuberculin. This latter finding appears to be strong evidence that disseminated calcifications are not frequently caused by tubercle bacilli, but probably by the agent producing sensitivity to histoplasmin.

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SPELEOTOMY

G. Le Carboulec devotes an exhaustive monograph (Paris 1945, Imprimerie Saint-Denis, Niort) to the technique of speleotomy, the "last word" in the surgical therapy of tuberculosis. This monograph covers current knowledge on the subject, the historical background, concepts of bronchial and cavitary anatomy, detection of cavities, operative and postoperative techniques, when the operation is indicated and its limits, and the results which have been obtained. The author reports on the experience of Bernou, leader of the Chateaubriant school, who has contributed, to a great extent, to the promotion of this type of surgical intervention which is still restricted to residual cavities under thoracoplasty. He concludes from his 21 observations that speleotomy should take a relatively important place in the treatment of cavities, when thoracoplasty and Monaldi's drainage have failed.

Aulanier and Liron describe the success of speleotomy in patients at the limit of operability (*Soc. d'et scient. de la tub.*, March 10, 1945).

DEATHS DURING WEEK ENDED DEC. 7, 1946

[From the Weekly Mortality Index, issued by the National Office of Vital Statistics]

	Week ended Dec. 7, 1946	Corresponding week, 1945
Data for 93 large cities of the United States:		
Total deaths	9,716	9,945
Average for 3 prior years	9,910	
Total deaths, first 49 weeks of year	441,814	439,644
Deaths under 1 year of age	761	640
Average for 3 prior years	631	
Deaths under 1 year of age, first 49 weeks of year	32,620	29,714
Data from industrial insurance companies:		
Policies in force	67,332,394	67,267,277
Number of death claims	11,963	13,085
Death claims per 1,000 policies in force, annual rate	9.3	10.1
Death claims per 1,000 policies, first 49 weeks of year, annual rate	9.4	10.0

INCIDENCE 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 DECEMBER 14, 1946

Summary

A total of 197 cases of poliomyelitis was reported for the week, as compared with 242 last week, 115 for the corresponding week last year, and a 5-year (1941–45) median of 86. Slight increases were reported in the New England, South Central, and Mountain areas, probably in most instances due to delayed reports. Of the 16 States reporting currently 5 or more cases, 9 reported an increase (63 to 87 cases), 5 showed a decline (87 to 50), while 2 showed no change. States reporting the largest number of cases are California 21, Illinois 18, New York and Texas 14 each, and Ohio and North Dakota 10 each. The cumulative total since March 16 is 24,489, as compared with 13,161 and 18,844, respectively, for the corresponding periods of last year and 1944, and a 5-year median for the period of 12,017.

Only slight increases were reported in the incidence of influenza. A total of 2,875 cases was reported, as compared with 2,813 last week and a 5-year median of 2,995. States reporting more than 100 cases are as follows (last week's figures in parentheses): Texas 1,365 (1,343), South Carolina 498 (423), Virginia 255 (422), Arizona 254 (261), Oklahoma 103 (15). The cumulative total since July 27 (approximate date of seasonal low for this disease) is 26,977, as compared with 240,750 for the corresponding period last year and a 5-year median of 27,484.

Four cases of psittacosis were reported in Michigan during the week.

Cumulative figures above those for last year for other diseases listed in the following table are Rocky Mountain spotted fever, tularemia, and undulant fever. The total to date for amebic dysentery is slightly above, but the cumulative totals for bacillary and undefined dysentery are below the corresponding figures for last year.

Deaths recorded during the week in 93 large cities of the United States totaled 9,612, as compared with 9,716 last week, 10,228 and 9,365, respectively, for the corresponding weeks of 1945 and 1944, and a 3-year (1943–45) average of 10,393. The total number recorded for these cities to date is 451,426, as compared with 449,872 for the corresponding period last year.

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Telegraphic morbidity reports from State health officers for the week ended Dec. 14, 1946, and comparison with corresponding week of 1945 and 5-year median

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

Division and State	Diphtheria			Influenza			Measles			Meningitis, meningococcus		
	Week ended—		Median 1941–45	Week ended—		Median 1941–45	Week ended—		Median 1941–45	Week ended—		Median 1941–45
	Dec. 14, 1946	Dec. 15, 1945		Dec. 14, 1946	Dec. 15, 1945		Dec. 14, 1946	Dec. 15, 1945		Dec. 14, 1946	Dec. 15, 1945	
NEW ENGLAND												
Maine	5	5	0	3	3	1	268	1	13	0	1	1
New Hampshire	0	0	0	1	149	1	55	34	4	0	0	0
Vermont	0	0	0	-	150	-	148	5	4	0	0	0
Massachusetts	25	10	5	-	-	-	239	152	152	3	3	6
Rhode Island	0	0	1	-	8	8	13	2	4	0	1	1
Connecticut	0	5	0	2	26	5	54	14	14	2	1	2
MIDDLE ATLANTIC												
New York	26	8	20	[1] 4	[1] 45	[1] 12	179	266	266	8	15	17
New Jersey	7	2	4	5	61	13	109	17	17	3	6	6
Pennsylvania	17	11	9	7	58	3	551	436	506	0	7	7
EAST NORTH CENTRAL												
Ohio	25	24	15	8	86	13	128	10	52	2	4	4
Indiana	23	7	3	4	595	15	11	3	21	3	2	1
Illinois	19	8	8	4	56	10	11	214	83	2	7	7
Michigan [2]	7	11	6	2	6	7	77	229	86	2	3	3
Wisconsin	9	3	2	22	388	49	28	24	129	0	1	3
WEST NORTH CENTRAL												
Minnesota	10	9	8	-	9	3	4	4	8	3	2	0
Iowa	1	10	3	2	65	-	7	6	44	2	0	0
Missouri	12	6	5	2	62	4	-	33	8	1	2	3
North Dakota	5	2	2	14	1, 244	12	-	-	2	1	0	0
South Dakota	3	0	2	-	2	-	3	1	3	0	0	0
Nebraska	2	2	2	-	86	31	3	3	12	2	0	0
Kansas	9	7	7	3	11, 229	48	4	41	41	1	0	2
SOUTH ATLANTIC												
Delaware	1	0	0	-	17	-	1	-	3	1	0	0
Maryland [2]	7	16	9	3	59	9	19	9	11	0	3	6
District of Columbia	0	0	0	-	22	3	14	2	2	0	0	0
Virginia	9	9	9	255	4, 691	236	34	64	64	3	4	4
West Virginia	4	3	5	49	3, 808	34	12	1	22	0	1	2
North Carolina	9	36	17	-	-	6	133	20	20	2	2	2
South Carolina	11	8	5	498	2, 659	460	60	13	13	0	1	1
Georgia	5	19	14	19	1, 000	80	27	12	21	2	0	1
Florida	15	11	8	20	8	8	22	4	4	0	0	1
EAST SOUTH CENTRAL												
Kentucky	24	11	5	-	89, 363	13	1	211	13	2	3	2
Tennessee	5	19	10	27	204	54	6	8	23	1	0	1
Alabama	19	25	17	44	649	98	34	2	2	1	4	1
Mississippi [2]	5	12	12	-	-	-	-	-	-	2	2	2
WEST SOUTH CENTRAL												
Arkansas	9	13	13	79	644	150	9	14	22	0	3	2
Louisiana	8	22	9	2	47	3	-	7	5	1	1	1
Oklahoma	8	6	9	103	684	137	2	5	8	2	0	0
Texas	16	74	58	1, 365	11, 259	1, 702	58	47	51	6	1	3
MOUNTAIN												
Montana	1	0	1	20	193	19	70	3	28	0	0	0
Idaho	0	0	0	8	279	1	2	73	11	1	1	0
Wyoming	0	1	0	-	66	66	1	10	8	0	0	0
Colorado	8	7	7	26	367	54	7	12	12	1	0	1
New Mexico	1	0	2	4	8	4	32	1	1	0	1	1
Arizona	5	7	2	254	1, 163	110	30	2	7	1	0	0
Utah [2]	0	0	0	2	17, 023	9	7	29	29	0	0	0
Nevada	0	0	0	-	-	-	-	-	1	0	0	0
PACIFIC												
Washington	3	5	7	-	226	12	23	220	52	0	1	3
Oregon	0	3	3	1	122	21	23	20	55	0	1	1
California	18	37	23	13	25	52	73	297	237	9	8	8
Total	396	474	416	2, 875	148, 914	2, 995	2, 592	2, 581	4, 425	70	92	108
50 weeks	15, 574	17, 746	14, 995	217, 174	309, 648	309, 648	658, 025	123, 670	587, 903	5, 535	7, 710	7, 710
	(27th) July 5–11			(30th) July 26–Aug. 1			(35th) Aug. 30–Sep. 5			(37th) Sep. 13–19		
	6, 946	10, 849	8, 508	26, 977	240, 750	27, 484	17, 940	21, 111	27, 635	869	1, 215	1, 215

[1] New York City only.
[2] Period ended earlier than Saturday.

Telegraphic morbidity reports from State health officers for the week ended Dec. 14, 1946, and comparison with corresponding week of 1945 and 5-year median—Con.

Division and State	Poliomyelitis			Scarlet fever			Smallpox			Typhoid and paratyphoid fever [4]		
	Week ended—		Median 1941–45	Week ended—		Median 1941–45	Week ended—		Median 1941–45	Week ended—		Median 1941–45
	Dec. 14, 1946	Dec. 15, 1945		Dec. 14, 1946	Dec. 15, 1945		Dec. 14, 1946	Dec. 15, 1945		Dec. 14, 1946	Dec. 15, 1945	
NEW ENGLAND												
Maine	0	1	0	37	24	24	0	0	0	3	0	1
New Hampshire	1	0	0	5	0	5	0	0	0	0	0	0
Vermont	3	1	1	3	10	2	0	0	0	0	0	0
Massachusetts	8	2	1	176	121	251	0	0	0	2	1	3
Rhode Island	1	1	0	11	12	12	0	0	0	0	0	0
Connecticut	1	1	1	28	29	40	0	0	0	0	0	0
MIDDLE ATLANTIC												
New York	14	16	8	306	296	301	0	0	0	2	2	5
New Jersey	4	0	1	70	46	85	0	0	0	0	1	1
Pennsylvania	3	0	1	169	174	194	0	0	0	5	2	4
EAST NORTH CENTRAL												
Ohio	10	2	2	257	326	269	1	0	0	2	2	2
Indiana	6	0	0	58	54	54	0	0	0	2	0	0
Illinois	18	14	2	118	145	164	0	0	0	1	1	2
Michigan [3]	6	3	1	161	209	189	0	0	0	0	3	3
Wisconsin	8	3	0	56	108	135	0	0	0	0	0	0
WEST NORTH CENTRAL												
Minnesota	4	0	1	37	42	67	0	0	0	0	0	0
Iowa	5	1	1	18	24	55	0	0	1	0	0	1
Missouri	4	7	1	33	59	59	1	0	0	0	1	1
North Dakota	10	0	0	11	8	13	0	0	0	3	0	0
South Dakota	5	1	1	4	4	29	0	0	0	2	0	0
Nebraska	6	0	1	19	22	27	0	0	0	0	0	0
Kansas	2	0	1	28	78	78	0	1	1	0	0	0
SOUTH ATLANTIC												
Delaware	0	0	0	7	3	5	0	0	0	1	0	0
Maryland [3]	2	0	0	35	46	46	0	0	0	2	1	1
District of Columbia	0	0	0	10	13	14	0	0	0	0	0	1
Virginia	1	3	1	42	94	65	0	0	0	2	0	0
West Virginia	0	0	0	23	47	47	0	0	0	0	0	0
North Carolina	1	2	2	33	61	67	0	0	0	1	0	0
South Carolina	0	1	1	9	8	12	0	0	0	0	4	1
Georgia	0	0	0	18	32	32	0	0	0	0	1	1
Florida	4	3	1	10	8	8	0	0	0	0	3	2
EAST SOUTH CENTRAL												
Kentucky	0	2	2	43	65	65	0	0	1	2	1	1
Tennessee	5	0	0	21	35	47	0	0	0	1	0	1
Alabama	2	2	0	8	30	23	0	2	0	2	1	1
Mississippi [3]	5	3	1	6	35	16	0	1	0	1	1	1
WEST SOUTH CENTRAL												
Arkansas	3	0	1	5	18	11	0	0	0	2	3	2
Louisiana	3	8	0	6	23	8	0	0	0	0	5	3
Oklahoma	2	0	0	11	18	22	1	2	2	0	3	1
Texas	14	4	3	33	105	55	0	0	0	2	9	6
MOUNTAIN												
Montana	0	2	0	8	9	19	0	0	0	3	0	0
Idaho	1	0	0	8	13	13	0	0	0	2	1	1
Wyoming	0	0	0	2	7	7	0	0	0	0	0	0
Colorado	1	0	0	33	43	35	0	0	0	0	0	0
New Mexico	2	0	0	14	17	8	0	0	0	0	0	1
Arizona	2	1	1	16	15	9	0	0	0	1	1	1
Utah [3]	0	0	0	31	29	32	0	0	0	0	2	0
Nevada	0	0	0	0	0	0	0	0	0	0	0	0
PACIFIC												
Washington	9	11	3	57	38	38	0	0	0	1	1	0
Oregon	0	5	0	30	34	34	0	0	0	1	0	0
California	21	15	9	143	245	171	0	0	0	3	3	4
Total	197	115	86	2,267	2,882	3,015	3	6	9	49	53	70
50 weeks	24,955	13,558	12,319	109,152	167,781	134,742	330	339	724	3,925	4,773	5,376
Seasonal low week [3]	(11th) Mar. 15–21			(32nd) Aug. 9–15			(35th) Aug. 30–Sep. 5			(11th) Mar. 15–21		
Total since low	24,489	13,161	12,017	22,857	33,963	33,963	51	66	108	3,450	4,149	4,791

[2] Period ended earlier than Saturday.
[3] Dates between which the approximate low week ends. The specific date will vary from year to year.
[4] Including paratyphoid fever reported separately, as follows: Maine 1; Massachusetts 2 (salmonella infection); New York 1; Ohio 2; Virginia 1; Arkansas 1.

Telegraphic morbidity reports from State health officers for the week ended Dec. 14, 1946, and comparison with corresponding week of 1945 and 5-year median—Con.

Division and State	Whooping cough			Week ended Dec. 14, 1946							
	Week ended—		Median 1941–45	Dysentery			Encephalitis, infectious	Rocky Mt. spotted fever	Tularemia	Typhus fever endemic	Undulant fever
	Dec. 14, 1946	Dec. 15, 1945		Amebic	Bacillary	Unspecified					
NEW ENGLAND											
Maine	18	40	39								
New Hampshire	25	12	11								
Vermont	12	20	20								2
Massachusetts	170	164	164								1
Rhode Island	16	24	24								
Connecticut	48	58	58		1						1
MIDDLE ATLANTIC											
New York	290	286	286	1	6		1				9
New Jersey	183	184	152								
Pennsylvania	274	129	129		1				1		10
EAST NORTH CENTRAL											
Ohio	112	119	119	6					6	1	5
Indiana	16	15	15			2	1		11		
Illinois	114	76	76	4			1		12		4
Michigan [2]	208	211	211						7		2
Wisconsin	273	93	177								8
WEST NORTH CENTRAL											
Minnesota	16	7	27	3							1
Iowa	18	19	19				1				15
Missouri	19	5	12						16		4
North Dakota		3	6				1				1
South Dakota		2	2			4					2
Nebraska	6	1	4								
Kansas	10	19	31						3		2
SOUTH ATLANTIC											
Delaware	1	5	5								
Maryland [3]	74	42	53					[3]	4		
District of Columbia	12	5	5						2		
Virginia	38	43	43			67			6		2
West Virginia	32	22	22								
North Carolina	76	46	89								
South Carolina	42	38	29		9				1	1	
Georgia	7	9	9	2	2				2	8	2
Florida	21	5	10				1		1	7	2
EAST SOUTH CENTRAL											
Kentucky	41	22	23					1	2		2
Tennessee	38	9	12						8		3
Alabama	50	21	15						1	5	3
Mississippi [2]									3		5
WEST SOUTH CENTRAL											
Arkansas	12	6	14				1		1		
Louisiana		2	2	3						2	6
Oklahoma	11	5	5	6					2		
Texas	216	139	139	10	241	55				7	12
MOUNTAIN											
Montana	3		10								1
Idaho	5	10	3								
Wyoming	6		6						1		
Colorado	16	14	14								1
New Mexico	24		3		1	4					
Arizona	13	6	10			58					
Utah [2]	1	13	19								1
Nevada											
PACIFIC											
Washington	30	49	49								1
Oregon	5	7	16	1							1
California	62	120	120	4	4		1				4
Total	2,664	2,125	2,125	40	265	190	8	1	90	31	123
Same week, 1945	2,125			50	434	165	7	1	36	76	64
Average, 1943–45	2,000			36	473	149	6	[4] 0	37	[4] 80	
50 weeks: 1946	96,419			2,350	16,007	6,297	600	[5] 569	1,052	3,294	5,161
1945	120,814			1,885	24,069	10,341	612	466	765	5,046	4,733
Average, 1943–45	129,040		[4]172,829	1,920	21,835	8,860	634	[4] 453	723	[4] 4,393	

[2] Period ended earlier than Saturday.
[3] Delayed report: Maryland, Rocky Mountain spotted fever, 1 October case.
[4] 5-year median, 1941–45.
Anthrax: Connecticut 1 case. *Psittacosis:* Michigan 4 cases.

WEEKLY REPORTS FROM CITIES [1]

City reports for week ended Dec. 7, 1946

This table lists the reports from 84 cities of more than 10,000 population distributed throughout the United States, and represents a cross section of the current urban incidence of the diseases included in the table.

Division, State, and City	Diphtheria cases	Encephalitis, infectious, cases	Influenza		Measles cases	Meningitis, meningococcus, cases	Pneumonia deaths	Poliomyelitis cases	Scarlet fever cases	Smallpox cases	Typhoid and paratyphoid fever cases	Whooping cough cases
			Cases	Deaths								
NEW ENGLAND												
Maine:												
Portland	0	0	------	0	55	0	0	1	3	0	0	5
New Hampshire:												
Concord	0	0	------	0	-------	0	0	0	0	0	0	------
Vermont:												
Barre	0	0	------	0	-------	0	0	0	0	0	0	------
Massachusetts:												
Boston	9	0	------	0	10	1	13	3	25	0	1	36
Fall River	0	0	------	0	1	0	1	0	1	0	0	1
Springfield	1	0	------	0	6	0	0	0	1	0	0	27
Worcester	0	0	------	0	-------	0	6	0	3	0	0	18
Rhode Island:												
Providence	0	0	2	0	11	0	1	0	7	0	0	15
Connecticut:												
Bridgeport	0	0	------	0	1	0	0	0	1	0	0	------
Hartford	0	0	------	0	-------	0	1	0	1	0	0	7
New Haven	0	0	------	0	17	0	1	0	0	0	0	------
MIDDLE ATLANTIC												
New York:												
Buffalo	2	0	------	0	-------	0	3	0	5	0	0	------
New York	28	1	4	1	22	2	41	14	55	0	1	38
Rochester	0	0	------	0	-------	1	2	0	8	0	0	2
Syracuse	0	0	------	0	-------	1	2	0	13	0	0	15
New Jersey:												
Camden	1	0	------	0	-------	0	2	0	0	0	0	4
Newark	0	0	2	0	3	0	8	0	5	0	0	29
Trenton	0	0	------	0	22	0	2	0	0	0	0	------
Pennsylvania:												
Philadelphia	4	0	6	2	4	2	14	1	26	0	0	39
Pittsburgh	2	0	------	0	261	0	12	0	15	0	0	11
Reading	0	0	------	0	1	0	2	0	0	0	0	6
EAST NORTH CENTRAL												
Ohio:												
Cincinnati	0	0	------	0	1	0	2	[2]0	9	0	1	8
Cleveland	0	0	4	1	67	1	5	2	16	0	0	7
Columbus	1	0	------	0	3	1	0	0	12	0	0	20
Indiana:												
Fort Wayne	0	0	------	0	-------	0	3	0	0	0	0	------
Indianapolis	3	0	------	0	2	0	4	0	7	0	0	14
South Bend	0	0	------	0	-------	0	0	0	5	0	0	------
Terre Haute	0	0	------	0	-------	0	0	0	0	0	0	------
Illinois:												
Chicago	1	0	------	0	4	1	19	9	50	0	0	39
Michigan:												
Detroit	5	0	1	0	6	0	11	1	36	0	0	61
Flint	0	0	------	0	-------	0	0	0	2	0	0	2
Grand Rapids	0	0	------	0	-------	0	1	0	11	0	0	12
Wisconsin:												
Kenosha	0	0	------	0	-------	0	0	0	1	0	0	------
Milwaukee	0	0	------	0	13	0	0	1	13	0	0	105
Racine	0	0	------	0	-------	0	0	1	6	0	0	16
Superior	0	0	------	0	-------	0	0	0	0	0	0	------
WEST NORTH CENTRAL												
Minnesota:												
Duluth	0	0	------	0	-------	0	0	1	0	0	0	4
Minneapolis	0	0	------	0	3	0	2	0	22	0	0	1
Missouri:												
Kansas City	1	0	------	0	-------	0	6	3	2	0	0	5
St. Joseph	0	0	------	0	-------	0	0	0	1	0	0	------
St. Louis	6	0	1	1	-------	0	16	2	8	0	0	7

[1] In some instances the figures include nonresident cases.

[2] Correction: Cincinnati, week ended November 2, poliomyelitis, 1 case (instead of 34). Rates: East North Central, 27.6; total, 23.9.

City reports for week ended Dec. 7, 1946—Continued

Division, State, and City	Diphtheria cases	Encephalitis, infectious, cases	Influenza		Measles cases	Meningitis, meningococcus, cases	Pneumonia deaths	Poliomyelitis cases	Scarlet fever cases	Smallpox cases	Typhoid and paratyphoid fever cases	Whooping cough cases
			Cases	Deaths								
WEST NORTH CENTRAL—continued												
Nebraska:												
Omaha	1	0	------	0	3	0	3	1	5	0	0	6
Kansas:												
Topeka	0	0	------	0	1	0	0	2	0	0	0	1
Wichita	0	0	------	0	------	0	3	0	3	0	0	2
SOUTH ATLANTIC												
Delaware:												
Wilmington	0	0	------	0	1	0	2	0	2	0	0	4
Maryland:												
Baltimore	20	0	------	0	5	1	10	0	13	0	0	41
Cumberland	0	0	------	0	4	0	0	0	0	0	0	------
Frederick	0	0	------	0	4	0	0	0	1	0	0	------
District of Columbia:												
Washington	0	0	------	0	------	3	5	2	2	0	0	4
Virginia:												
Lynchburg	0	0	------	0	------	0	0	0	0	0	0	------
Richmond	0	0	1	1	6	0	5	0	2	0	0	------
Roanoke	0	0	------	0	------	0	0	0	1	0	0	------
West Virginia:												
Wheeling	0	0	------	0	------	0	0	0	0	0	0	1
North Carolina:												
Raleigh	0	0	------	0	------	0	2	1	0	0	0	4
Wilmington	1	0	------	0	3	0	2	0	0	0	0	------
Winston-Salem	0	0	------	0	37	0	0	0	0	0	0	1
South Carolina:												
Charleston	0	0	5	0	------	0	0	0	0	0	0	------
Georgia:												
Atlanta	1	0	2	1	7	0	2	0	2	0	0	5
Brunswick	0	0	------	0	------	0	1	0	0	0	0	------
Savannah	0	0	------	0	7	0	0	0	1	0	0	------
Florida:												
Tampa	3	0	------	0	------	0	1	0	0	0	0	------
EAST SOUTH CENTRAL												
Tennessee:												
Memphis	1	0	------	2	3	0	4	2	0	0	0	12
Nashville	0	0	------	2	------	0	3	0	1	0	0	------
Alabama:												
Birmingham	1	0	3	1	1	0	3	0	2	0	0	------
Mobile	4	0	2	1	------	0	0	0	0	0	1	1
WEST SOUTH CENTRAL												
Arkansas:												
Little Rock	2	0	------	0	4	0	0	0	2	0	0	------
Louisiana:												
New Orleans	0	0	1	1	4	0	5	1	1	0	0	1
Shreveport	0	0	------	0	------	1	6	2	0	0	1	------
Texas:												
Dallas	2	0	------	0	3	0	2	1	1	0	0	1
Galveston	0	0	------	0	------	0	1	0	0	0	0	------
Houston	0	0	------	0	------	1	2	2	1	0	0	------
San Antonio	0	0	------	0	1	0	8	0	0	0	0	6
MOUNTAIN												
Montana:												
Great Falls	0	0	------	0	2	0	0	0	0	0	0	2
Helena	0	0	------	0	------	0	0	0	0	0	0	------
Missoula	0	0	------	0	------	0	2	0	0	0	0	1
Colorado:												
Denver	1	0	8	0	2	1	2	0	13	0	0	5
Pueblo	0	0	------	0	------	0	1	0	0	0	0	------
Utah:												
Salt Lake City	0	0	------	0	4	0	2	0	4	0	0	------

City reports for week ended Dec. 7, 1946—Continued

Division, State, and city	Diphtheria cases	Encephalitis, infectious, cases	Influenza		Measles cases	Meningitis, meningococcus, cases	Pneumonia deaths	Poliomyelitis cases	Scarlet fever cases	Smallpox cases	Typhoid and paratyphoid fever cases	Whooping cough cases
			Cases	Deaths								
PACIFIC												
Washington:												
Seattle	1	0	------	0	3	1	8	0	4	0	0	9
Spokane	0	0	1	0	3	1	3	0	3	0	0	------
Tacoma	0	0	------	0	------	0	0	0	2	0	0	------
California:												
Los Angeles	4	0	3	0	7	2	4	4	24	0	0	16
Sacramento	0	0	------	0	------	0	0	0	4	0	0	3
San Francisco	0	0	1	0	3	2	6	1	7	0	0	------
Total	106	1	47	14	631	23	278	58	471	0	5	680
Corresponding week, 1945	64	------	350	42	781	------	385	------	681	0	8	637
Average, 1941–45	88	------	856	[3] 73	[4] 736	------	[3] 487	------	846	0	13	721

[3] 3-year average, 1943–45.
[4] 5-year median, 1941–45.
Dysentery, amebic.—Cases: New York 5; Chicago 1; Nashville 2.
Dysentery, bacillary.—Cases: New York 1; Detroit 1; Los Angeles 3.
Dysentery, unspecified.—Cases: San Antonio 8.
Tularemia.—Cases: Cincinnati 1; Cleveland 1; Indianapolis 1; Chicago 1; St. Louis 4; New Orleans 1.
Typhus fever, endemic.—Cases: Tampa 2; Nashville 1; Birmingham 3; New Orleans 2; Dallas 1; Los Angeles 2.

Rates (annual basis) per 100,000 population, by geographic groups, for the 84 cities in the preceding table (estimated population, 1943, 33,891,000)

	Diphtheria case rates	Encephalitis, infectious, case rates	Influenza		Measles case rates	Meningitis, meningococcus, case rates	Pneumonia death rates	Poliomyelitis case rates	Scarlet fever case rates	Smallpox case rates	Typhoid and paratyphoid fever case rates	Whooping cough case rates
			Case rates	Death rates								
New England	26.1	0.0	5.2	0.0	264	2.6	60.1	10.5	110	0.0	2.6	285
Middle Atlantic	17.1	0.5	5.6	1.4	145	2.8	40.7	6.9	59	0.0	0.5	67
East North Central	6.1	0.0	3.1	0.6	59	1.8	27.6	8.6	103	0.0	0.6	174
West North Central	18.0	0.0	2.3	2.3	16	0.0	67.6	20.3	92	0.0	0.0	59
South Atlantic	41.9	0.0	13.4	3.3	124	6.7	50.2	5.0	40	0.0	0.0	100
East South Central	35.4	0.0	29.5	35.4	24	0.0	59.0	11.8	18	0.0	5.9	77
West South Central	11.5	0.0	2.9	2.9	34	5.7	68.9	17.2	14	0.0	2.9	23
Mountain	8.5	0.0	68.4	0.0	68	8.5	59.8	0.0	145	0.0	0.0	68
Pacific	7.9	0.0	7.9	0.0	25	9.5	33.2	7.9	70	0.0	0.0	44
Total	16.4	0.2	7.3	2.2	97	3.5	42.9	8.9	73	0.0	0.8	105

TERRITORIES AND POSSESSIONS

Hawaii Territory

Plague (rodent).—Under date of December 9, 1946, rodent plague infection was reported on September 20, 1946, in District 14B, Makawao, Island of Maui, T. H.

FOREIGN REPORTS

CANADA

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

Disease	Prince Edward Island	Nova Scotia	New Brunswick	Quebec	Ontario	Manitoba	Saskatchewan	Alberta	British Columbia	Total
Chickenpox		16		246	382	21	34	72	99	870
Diphtheria			2	42	11	3	1	7	2	68
Dysentery:										
Amebic					8					8
Bacillary				1						1
German measles					8	1	1	5	5	20
Influenza		15			3		1		13	32
Measles		219		94	56	20	217	117	78	801
Meningitis, meningococcus				1	6		1	1	2	11
Mumps				60	242	31	65	27	125	550
Poliomyelitis		1		7	10					18
Scarlet fever		6	15	139	98	9	3	7	8	285
Tuberculosis (all forms)		4	8	112	74	21	5	12	80	316
Typhoid and paratyphoid fever				18	5	1			2	26
Undulant fever				1						1
Venereal diseases:										
Gonorrhea		12	8	182	126	34	41	33	78	514
Syphilis	1	20	7	84	91	28	20	12	49	312
Other forms				1					2	3
Whooping cough		11		55	71	11	5	3	30	186

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

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

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

Plague

Madagascar.—For the period November 11–20, 1946, 10 cases of plague were reported in Madagascar.

Palestine—Jaffa.—On December 2, 1946, 1 fatal case of plague was reported in Jaffa, Palestine.

Peru.—During the month of October 1946, 19 cases of plague with 2 deaths were reported in Huancabamba Province, Piura Department, and 1 case of plague was reported in Chancay Province, Lima Department, Peru.

Smallpox

Malay States (Federated).—For the week ended December 7, 1946, 262 cases of smallpox were reported in the Federated Malay States.

Venezuela.—For the week ended November 30, 1946, 157 cases of smallpox (alastrim) were reported in Venezuela, including 131 cases reported in Sucre State, 7 cases reported in Anzoategui State, 7 cases reported in Aragua State, and 12 cases reported in Cojedes State.

Typhus Fever

Eritrea.—Typhus fever has been reported in Eritrea as follows: Weeks ended—November 16, 1946, 59 cases, 2 deaths; November 23, 1946, 85 cases, 10 deaths.

Yellow Fever

French Equatorial Africa—Carnot.—On December 7, 1946, 4 cases of yellow fever among the natives were reported confirmed in Carnot, French Equatorial Africa.

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