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INVESTIGATION ON SOIL POLLUTION AND THE RELATION OF THE VARIOUS TYPES OF PRIVIES TO THE SPREAD OF INTESTINAL INFECTIONS

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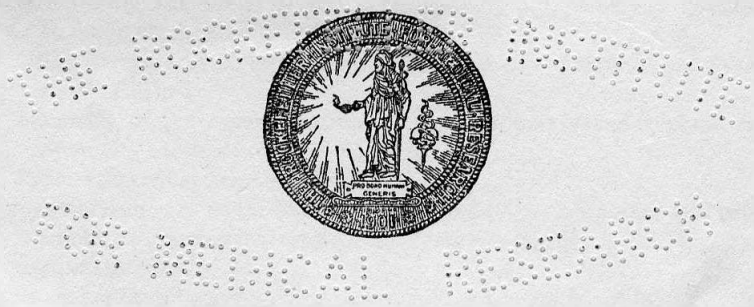

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INVESTIGATION ON SOIL POLLUTION AND THE RELATION OF THE VARIOUS TYPES OF PRIVIES TO THE SPREAD OF INTESTINAL INFECTIONS.*

BY I. J. KLIGLER, PH.D.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

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INTRODUCTION.

The safe disposal of human excreta is one of the most serious problems confronting the rural sanitarians. They all realize that the control of enteric infections is intimately bound up with the success in rendering human excreta innocuous. This task is not, however, easily accomplished in the rural community because of the difficulty of devising a safe and practical method of disposal for the individual home. In recent years, a number of devices have been proposed and endorsed, but none of them has received general approval.

The methods of disposal usually employed in rural communities fall into four main types: (*a*) the open back privy; (*b*) the pit privy; (*c*) the pail type; and (*d*) the septic tank. Occasionally the chemical closet is also used. Some of these so called sanitary closets are unquestionably dangerous; others have their respective advantages and degrees of usefulness. None of them can reasonably be considered as satisfactory as the urban sewerage system method of disposal. But the potential or actual danger involved in the use of one or another type is purely a matter of conjecture.

A general comparison of the types of privies mentioned reveals certain relative merits and demerits. The *open back privy*, which consists merely of a seat, with or without a shed, open in the rear for the removal of accumulated excreta, is obviously dangerous. The fecal material is constantly exposed to flies, rain wash, animal distribution, etc. It constitutes an advance over the no closet stage and,

at any rate, the material is concentrated at a single point instead of being disposed over a wider area. The *pit privy* marks a distinct advance over the open back type. The excreta are concentrated in a single hole and, if the seat fits tightly over the pit, it is comparatively free from flies. But although the possibility of fly distribution and spread by surface wash are greatly reduced, there remains the possible danger of subsoil pollution of the ground-water. The *pail* type represents an attempt to establish a privy free from any of the objections that apply to the pit privy. The excreta are collected into tight pails which are periodically collected and emptied. Theoretically it ought to be entirely satisfactory. Practically it falls far short of attaining the ideal. It requires too much attention, scavengers are careless, and it is not always fly-tight.

The septic privy, or the *wet* system, is a definite departure from the *dry* types of closets described above. In all these types the liquefying bacteria are depended upon to disintegrate the solid feces and destroy the pathogens. The primitive leaching cesspool may be considered the empirical prototype. The main representatives of the modern varieties are the Lumsden-Roberts-Stiles (L. R. S.) privy and the Kentucky sanitary privy, of which there are several modifications in actual use. The L. R. S. privy consists of two barrels connected with a siphon, one receiving the excreta and constituting the septic chamber, the other receiving the effluent. The great objection to this system lies in the fact that the effluent in the second chamber must be frequently disposed of to prevent overflow. The Kentucky sanitary privy is an attempt to overcome the latter objection. This closet is a concrete three-chamber arrangement: a septic chamber divided into two compartments by a baffle-board, and an effluent chamber. In this device the effluent is disposed of by subsurface irrigation through unglazed tiling. This closet requires little or no attention, but again one is confronted with the possibility of subsoil pollution of the ground-water.

It is evident, then, from this brief résumé of the various types of sanitary privies in more or less general use, that most of them and particularly the two requiring least attention—the pit and the Kentucky sanitary privy—are a possible menace because of their likelihood of polluting the ground-water by way of the subsoil. This possibility

has, however, been purely conjectural, and the relative danger of such pollution actually occurring remains to be determined.

The question of the relation of soil pollution to the spread of infectious diseases is an old one. No one has, however, actually succeeded in proving that such a direct relation existed. In the early days of bacteriology Bowditch in this country and Buchanan in England associated the spread of certain diseases, particularly tuberculosis, with dampness of soil. Pettenkoffer formulated a definite relationship between the condition of the soil and the occurrence of enteric fever. The conditions which he considered favorable to an enteric epidemic were: (*a*) a rise and rapid fall of the ground-water; (*b*) pollution of the soil; (*c*) a certain ground temperature; and (*d*) a specific organism. Ballard (1887) also associated summer diarrhea with a definite soil temperature, the pollution of the soil with organic matter, and a specific microorganism. His conclusions in his report to the Local Government Board[1] are of interest, because they are illustrative of the views held in the early days of the development of bacteriology.

(*a*) The essential cause of diarrhea resides ordinarily in the superficial layers of the earth, where it is intimately associated with the life processes of some microorganism not yet detected or isolated.

(*b*) The vital manifestations of such an organism are dependent, among other things, perhaps principally, upon the conditions of the season and the presence of dead organic matter, which is its pabulum.

(*c*) Such a microorganism is capable of getting abroad from its primary habitat, the earth, and, having become air-borne, obtains opportunity for fastening on non-living organic material and of using such organic matter both as nidus and as pabulum in undergoing various phases of its life history.

(*d*) From food, as also from contained organic matter of particular soils, such a microorganism can manufacture, by the chemical changes wrought therein through certain of its life processes, a substance which is a virulent chemical poison.

In the last three decades the question has been approached from the more exact bacteriological view-point, but definite proof of the direct relation between soil contamination and the spread of infection is meager. The work of Looss, Bentley, Stiles, and others has established conclusively the relation between soil pollution and hookworm

[1] Ballard, *Rep. Med. Off., Local Gov. Bd.*, 1887, suppl., 7.

infection. According to Stiles the hookworm larvæ may survive in sand cultures for 117 days and in sand under and around the privy for about 5 months. Looss (1903) showed experimentally that infection may occur through the skin. A similar relation between soil pollution and intestinal diseases of bacterial origin has not as yet been shown to exist. There is, of course, the possibility of the distribution of infectious microorganisms from polluted soil by means of flies or water. The actual danger that the soil will become infected or that the infectious material will find its way from the subsoil to the surface or to the water supply has not been definitely ascertained.

The purpose of this investigation was, therefore, to study the various types of privies, particularly with regard to the danger of soil pollution and its relation to the spread of intestinal infections. Special attention has been given to the pit and septic types of privy, though other varieties, particularly the pail and chemical closets, were also studied.

The problem has been approached both from the experimental and practical standpoint. In the laboratory repeated tests have been made to determine: (1) the viability of the typhoid and dysentery bacilli in soil and in excrement under different conditions; (2) their ability to penetrate through columns of soil of different porosity; (3) their viability in septic fluids and effluents; and (4) the nature of the antagonistic factors in soil and septic material which influence the viability of these microorganisms. In the field work various types of privies of different ages were examined particularly with regard to (1) the extent of pollution of the soil surrounding these privies; (2) their relation to well pollution; and (3) the passage of material from the privies through the soil to adjoining wells.

The main conclusion arrived at on the basis of both the experimental and field observations is *that in moderately compact clay, sand-clay, or sandy soil, free from cracks, the possibility of subsoil pollution of the ground-water is negligible, provided the ground-water level is more than 10 feet below the polluted area.* This statement is a deduction from the various subsidiary conclusions which will be discussed in detail in the course of this report and should be accepted only in connection with the modifying conditions that controlled the various tests and experiments.

PREVIOUS INVESTIGATIONS.

The generalizations of Pettenkoffer and Ballard which have been referred to were followed by more exact investigations bearing on the question of soil pollution and its relation to infection. These investigations bearing directly or indirectly on the question may best be summarized under certain subheadings.

Persistence of Bacillus typhosus and Bacillus dysenteriæ in Soil.

With few exceptions the studies reported in the literature bear only indirectly on the general problem. Most authors have confined themselves to noting the persistence of pure cultures of intestinal pathogens and parasites (usually *B. typhosus* and *B. coli*) in soil under diverse conditions. Houston, Chick, Smith, Horrocks, and others found that *B. coli* is present but rarely in uncultivated soil, but when added to soil the organism may persist for from 6 to 8 weeks. With regard to *B. typhosus*, the results are more conflicting. Grancher and Deschamps (1889) recovered this bacillus 5½ months after it was mixed with soil contaminated with organic matter. Karlinski (1889) found that *B. typhosus* survived for 3 months in natural soil. Dempster (1894), on the other hand, found it in sterile sand after 23 days, in garden mold after 42 days, in dry soil only up to the 7th day, and in peaty soil not after 24 hours. He concluded that moisture is the important factor, the organism remaining alive much longer in moist than in dry soil. Pfuhl (1902) likewise found that *B. typhosus* survived for 88 days in moist garden soil and for only 28 days in dry soil, and that *B. dysenteriæ* behaved similarly, remaining alive for 101 days in moist soil and for only 12 days in dry soil. Robertson (1898) reports that when organic substances (diluted bouillon) were added to unsterilized soil, the organisms maintained themselves for 315 days, or from summer to summer, while in natural soil they were isolated after 86 days. Martin conducted an extensive series of experiments over a period of several years (1898–1901) with various kinds of soil under different conditions. In these studies he found that in sterile, cultivated garden soil at a temperature between 3° and 19°C., *B. typhosus* remained alive for 404 days, while in sterile, virgin soil it died out rapidly. In sterile peat soil it was not recovered after 24 hours. In wet, unsterilized, cultivated soil it disappeared very rapidly (2 days); in similar soil, somewhat drier and kept at 2–12°C., it persisted for 12 days; in sterile soil under the same conditions it was recovered up to the 57th day. Savage (1905) found that *B. typhosus* will survive in tidal mud at least 2 weeks, and sometimes as long as 35 days. Firth and Horrocks (1902), in a series of experiments under approximately natural conditions, found that laboratory strains of *B. typhosus* could be recovered from unsterilized soil at 48°F. for 55 days, while freshly isolated strains disappeared after 32 days. In dry soil they remained alive for about 4 weeks. They agree with the view of Dempster and Pfuhl that moisture

is an important factor, and that the bacilli persisted for longer periods in damp than in dry soil. Rullmann (1901, 1905) isolated *B. typhosus* from unsterilized soil, receiving organic fluids after 100 days; while in contaminated sterile soil it was found after 16 months. Sedgwick and Winslow (1902) conclude that in dry earth typhoid bacilli die out rapidly, only a fraction of 1 per cent persisting

TABLE I.

Survival of Bacillus Typhosus in Soils.

	Cultivated soil.				Natural soil.				Peat.
	Sterile.		Non-sterile.		Sterile.		Non-sterile.		
	Moist.	Dry.	Moist.	Dry.	Moist.	Dry.	Moist.	Dry.	
	days		*days*		*days*		*days*	*days*	*days*
Grancher and Deschamps...			165						
Karlinski...							90		
Dempster...					23		42	7	1
Pfuhl.....							88	28	
							(*B. dysenteriæ*)		
							101	12	
Robertson...			315*				86		
Martin.....	404		Very wet, 2. Damp, 12.		57		1 (?)		1
Firth and Horrocks..			74				55	22	
							32	30	
							67	25	
							44		
Rullmann...	480		100*						
Mair.....					89		74		
					69		69		
					72		42		
							50		
Schmidt....							(*B. dysenteriæ*), winter.		

* Broth added to soil.

after 2 weeks, whereas in moist earth the destruction is less rapid. Cold alone is not an important factor. Smith (1904), without giving details of his experiments, concludes that the typhoid bacillus persists for only a short period in natural soil and for a somewhat longer time in sterile soil. Mair (1908), on the contrary, found that in garden soil at 20°C. *B. typhosus* survived for from 42 to 74 days in the unsterilized, and for only 9 days in the sterile soil. Schmidt (1902) claims that *B. dysenteriæ* survived all winter in damp soil.

It is difficult, on account of the many factors involved, such as the character of the soil, moisture, temperature, etc., to harmonize these divergent results. On tabulating the data obtained by the various investigators (Table I) with regard to the special conditions of the experiment, a general agreement on certain main points is found. The typhoid bacillus (and apparantly also the dysentery bacillus (Pfuhl)), will survive longer in wet than in dry soil, irrespective of whether the soil is sterile or not. The viability varies according to the different authors from 42 to 90 days for the damp soil and from 12 to 30 days for the dry soil. These variations are not excessive when the resistance of individual strains, the effect of reaction of the soil, and other modifying conditions which these investigators entirely overlooked are kept in mind. Peaty soil is evidently highly destructive to these bacteria. The claim of some authors (Rullmann, Martin), that the typhoid bacillus persists longer in sterile than in non-sterile soil, is not conclusively proved and is contested by Mair. Martin attributes the different results in sterile and non-sterile soil to the antagonistic action of soil bacteria, while Mair believes that they are simply due to overgrowth. The findings are too divergent and the experiments too few in number to warrant a definite conclusion, but they indicate that the addition of sterile bouillon tends to prolong the life of the bacteria, and that the viability is greater in sterile than in natural soil. The condition imposed is, however, entirely artificial and has only a limited bearing on the question.

It should be noted that all these experiments were conducted with pure cultures grown on artificial media. It may be questioned whether these results are directly applicable to bacilli deposited on the soil in fecal matter. As a rule, freshly isolated strains are less resistant than old stock cultures. Houston's work (1900–02) on the viability of sewage bacteria on soil to which sewage has been added throws some light on this point. His conclusions from his experiments are briefly as follows.

(1) The addition of sewage to an ordinary garden soil does not seem to lead to other than a temporary increase in sewage bacteria; the ordinary soil bacteria soon outgrow the sewage organisms. (2) The addition of sewage to sandy soil leads to an enormous increase in the total number of organisms, and the bacterial flora does not revert to its original condition for some months. (3) The addition of sewage to garden soil leads to a temporary increase in the ratio of the total number of bacteria to aerobic spores. (4) The addition of sewage to soil increases for a time the number of gas-forming bacteria—*B. coli* and *B. sporogenes*—and streptococci, but these die out within a month or two.

It is evident from Houston's experiments that there is a keen struggle between the natural soil flora and the foreign organisms, and that the former soon gain the upper hand.

Persistence of Bacillus typhosus and Bacillus dysenteriæ in Fecal Discharge.

The investigations on the survival of pathogenic bacteriạ in excrement are of special interest in connection with our problem. These experiments are not numerous. Karlinski (1889) found *B. typhosus* in pit material on the 3rd day and in feces after 3 months. Levy and Kayser (1903) claim to have isolated this bacillus from infected stools kept in a dark pit after 5½ months. Gärtner (1898) found that *B. typhosus* remained alive in stools from 3 to 10 days. Park (1907) also found that the typhoid bacillus may be recovered from feces up to the 10th day. Fürbringer and Stietzel (1908) obtained positive cultures from privy material inoculated with *B. typhosus* after 4 weeks, 48 days, and 52 days, respectively. Galvagno and Calderini (1908), in experiments conducted under natural conditions, found that *B. typhosus* could be isolated from pits containing a mixture of typhoid and normal stools in from 15 to 30 days and if the excrement is spread on damp soil the bacilli persist 10 days longer. According to Kruse (1901) the dysentery bacilli are rarely found in feces after 2 days.

It appears, therefore, that the typhoid bacillus may survive in excrement under various conditions at least 10 to 30 days, while the dysentery bacilli persist for much shorter periods.

Viability of Bacillus typhosus, Etc., in a Septic Tank.

Since the septic tank is used for the disposal of excrement, it is, of course, important to know how long pathogenic bacteria will persist under those conditions. Rideal (1901), in his Report from the Commissioners of Great Britain, states that Pickard has shown that typhoid bacilli introduced into a septic tank die out gradually until the 14th day, when only 1 per cent remains alive. Experiments conducted by the Royal Commission for Sewage Disposal showed that when partially sterilized effluents from the Cameron bed are inoculated with *B. typhosus*, there is a reduction of over 99 per cent in the first 6 days, and practically complete destruction in 10 days. In unsterilized effluents the rate of reduction is practically the same. Laws and Andrewes (1894) found that *B. typhosus* perished quickly in either filtered or heat-sterilized sewage. Horrocks (1899) found that typhoid bacilli will persist in sterile sewage for 60 days but could be recovered only up to the 5th day if *B. coli* was inoculated at the same time. MacConkey (1902) recovered *B. typhosus* after 17 days from sterilized crude sewage and after 13 days from non-sterile sewage. Russell and Fuller (1906) report that typhoid bacilli placed in collodion sacs and immersed in sewage died out after 5 days. Finally, Eijken and Grijns (1917), in the course of an investigation which will be discussed more fully later, found that *B. typhosus* persisted in septic

material for a period of from 3 to 7 days. The general consensus, then, seems to be that typhoid bacilli may survive in septic material for as long as 14 days, but that the larger proportion of them die out in the first 3 to 6 days.

Penetration of Bacteria through Soil.

The capacity of bacteria to penetrate through soil of different degrees of compactness is of even greater significance for our problem than their ability to survive in it. If the organisms that persist remain where they are placed, the danger of dissemination of disease is slight. On the other hand, if they are able to spread readily through soil the menace becomes real. Only a few inconclusive studies have been reported dealing with this question. A number of investigators used chemical substances, and their results, though interesting, can hardly be applied to bacteria. The experiments by McCallie (1904) with salt, and by Dole (1906), Marboutin (1901), Martel (1903), and others with fluorescine indicate the capacity of these substances to penetrate certain soils for considerable distances. These results cannot, however, be translated into terms of living cells except perhaps where the findings are negative. A careful experiment recently reported by Tanner and Bartow (1916) is of interest. They attempted to trace the source of the pollution of a well. To do this they distributed a ton of fine salt among eleven vaults surrounding the well and within 200 to 300 feet from it. The chlorine content of the well water was then tested daily for over a month, but no increase was noted.

A number of investigators, realizing the fallacy of applying the data of chemical penetration to bacteria, attempted to determine directly the ability of certain bacteria to pass through soil. Pfuhl (1897) used *B. prodigiosus* and was able to show that when added to an experimental pit in gravel soil this bacillus could be washed through a depth of 8 meters in 2 hours. Abba and his collaborators (1899), working with natural soil, found that this organism may be washed in 48 hours by prolonged rain through 2 to 3 meters of soil into a water supply 200 meters away. Under ordinary conditions, however, the bacillus will remain in the soil for a long time without appearing in the water supply. Grancher and Deschamps (1889) were unable to wash *B. typhosus* through 8 feet of soil. Firth and Horrocks (1902) using various sized cylinders filled with soil, found that *B. typhosus* could be washed through a column of 18 inches by an artificial rain falling at the rate of 2.2 inches per hour, if allowed to fall for 3 hours, but could not be washed through a column of 2 feet by 3.5 inches per hour of rain falling for 5 hours. In no instance did they observe the spreading of the bacilli either upward or laterally. Robertson (1898) likewise could not recover *B. typhosus* at distances of 1, 3, and 9 feet, respectively from his inoculated experimental patch. Experiments conducted by Davies and Tyndale (1905) with sewage in a limestone region showed that when sewage is applied on the surface the flow is perpendicularly downward with no lateral diffusion. When the surface was deluged with sewage,

it did pass through a depth of 9 feet, but as ordinarily applied, there was no penetration other than soakage. While the data bearing on this subject are meager, it appears that there is no penetration of bacteria in a lateral or upward direction in ordinary soil, but that they may be washed downward by heavy rains to a depth of at least 18 inches and sometimes even 6 feet in moderately compact soil, and to greater depths in gravel and limestone soil. It is evident, however, that this question requires further study.

EXPERIMENTAL.

The present investigation extended over a period of 2 years, the problem being approached from the field and experimental viewpoints. In the course of the first year, attention was devoted in the laboratory to improvements of technique, and in the field to the study of the pit privy and its relation to soil and well pollution. During the second year the question of viability of bacteria in soil, feces, pit, and septic material, and the factors controlling it were studied in the laboratory, while in the field the septic privy and an experimental pit formed the subjects of investigation. In the course of these inquiries some attention was also given to the pail and chemical types of closets, but neither of them was studied as intensively as the pit and septic privies.

The present researches naturally assumed two phases, the laboratory experiments on the one hand, and the field studies on the other. It appears, therefore, logical to present the results of these studies independently and then attempt to correlate them.

LABORATORY EXPERIMENTS.

Viability of B. typhosus and B. dysenteriæ in Fecal Matter.—Although there are data in the literature bearing on this point, it seemed desirable to repeat the tests in order to confirm and possibly extend previous observations. Typhoid carrier stools as well as normal stools mixed with 24 hour broth cultures of typhoid and dysentery bacilli, respectively, were used in testing the viability. In order to determine the effect of the condition of the stool, tests were made with liquid and solid emulsions. The stools were kept in cork-stoppered vials of about 2 ounce capacity at laboratory temperature, ranging from 68–75°F. (20–24°C.). Samples of the feces were examined at 24 or 48 hour intervals, and the tests were discontinued after three consecutive negative tests. The results are summarized in Table II.

It is evident from these results that the typhoid bacilli may remain alive in the stools in sufficient numbers to be recovered up to about the 10th day. They appear to survive longer in solid than in liquid feces. In only one stool were the bacilli recovered on the 15th day,

TABLE II.

Viability of Typhoid and Dysentery Bacilli in Fluid and Solid Feces.

Organism.	Nature of stool.	Date started.	Last date positive.	Viability.
				days
B. typhosus.	Carrier, solid.	Oct. 23	Nov. 1	9
B. "	" "	" 23	" 1	9
B. "	" "	Nov. 6	" 13	7
B. "	Normal emulsion, solid.	" 17	" 27	10
B. "	Carrier, solid.	" 27	Dec. 12	15; kept on ice, Nov. 27 to Dec. 1.
B. "	Normal emulsion, solid.	Mar. 30	Apr. 6	6
B. "	" " "	" 30	" 8	8
B. "	" " "	" 30	" 8	8
B. "	" " fluid.	" 29	" 4	6
B. "	" " "	" 29	" 2	4
B. "	" " "	" 29	" 1	3
B. dysenteriæ Flexner.	" " solid.	Nov. 17	Nov. 20	3
B. " "	" " "	Dec. 1	Dec. 4	3
B. " "	" " "	" 4	" 12	8
B. " "	" " "	Mar. 30	Apr. 5	5
B. " "	" " "	" 30	" 4	4
B. " "	" " "	" 30	" 5	5
B. " "	" " fluid.	" 29	" 1	3
B. " "	" " "	" 29	Mar. 30	1
B. " "	" " "	" 29	Apr. 1	3
B. dysenteriæ Shiga.	" " solid.	" 30	" 2	2
B. " "	" " "	" 30	" 1	1
B. " "	" " "	" 30	" 2	2
B. " "	" " fluid.	" 29	Mar. 30	1
B. " "	" " "	" 29	" 30	1
B. " "	" " "	" 29	Apr. 1	2

but in that instance the stool was kept for 4 days in the ice box at a temperature of 4°C. before being placed at room temperature. Storage at a low temperature (4°C.) apparently tends to preserve the bacilli and does not impair their viability on subsequent exposure to room temperature. Dysentery bacilli of the Flexner type can be

recovered from feces up to from 3 to 8 days, while the Shiga bacillus was isolated on the 2nd day but not later. The dysentery bacilli survived longer in solid than in liquid stools. Since freshly isolated strains are more sensitive to exposure, it may be assumed that they would not survive longer than the old stock strains. These findings are entirely in accord with those reported by Gärtner and by Park.

Viability of Bacillus typhosus, Bacillus paratyphosus, and Bacillus dysenteriæ Flexner in Pit Material.—In order to extend the observations recorded in the foregoing paragraphs and to render the results more nearly applicable to actual pits, the following experiments were made.

TABLE III.

Viability of Typhoid, Paratyphoid, and Dysentery Bacilli in Pits.

Jar.	Culture.	Pit started.	Tests started.	Date negative.	No. of samples.	Period of survival.
						days
1	*B. typhosus.*	Nov. 7, 1917	Dec. 24, 1917	Jan. 2, 1918	4	<10
	B. "	Jan. 8, 1918	Feb. 4, 1918	Feb. 11, 1918	3	< 7
2	*B. paratyphosus* B.	Nov. 22, 1917	Dec. 24, 1918	Jan. 8, 1918	4	<15
	B. " "	Jan. 10, 1918	Feb. 11, 1918	Feb. 25, 1918	4	<15
3	*B. dysenteriæ* Flexner.	Nov. 22, 1917	Dec. 24, 1917	Dec. 27, 1917	4	< 3
	B. " "	Jan. 3, 1918	Feb. 8, 1918	Feb. 12, 1918	3	< 4

A series of glazed crocks was employed as pits. They were covered with copper gauze to keep out insects, and kept in a dark place at a temperature ranging between 20–25°C. These pits were used continually over a period of 4 or 5 weeks. At weekly intervals typhoid carrier stools or fecal emulsions of paratyphoid or dysentery bacilli were added to the respective crocks. Dry powdered soil was added to these pits after each movement to keep them moderately dry. At intervals the use of the jars was discontinued and daily tests were made for the presence of the respective bacilli—typhoid, paratyphoid, or dysentery. After two or three consecutive negative tests were obtained, the crocks were again put into use and treated in the same manner as previously outlined. Two series of tests were made on each jar.

The results of these tests are given in Table III.

The tests on these artificial pits show that under the conditions of the experiment the typhoid bacilli were not recovered after 8 days, the paratyphoid B bacilli not after 15 days, and the dysentery bacillus not after 4 days. These survival periods correspond with those found

in feces, the repeated inoculations apparently not altering the effect. The results are lower than those obtained by Galvagno and Calderini, who were able to recover the typhoid bacillus in 15 to 20 days. The discrepancy may be due to the difference in moisture. In the present experiments, the soil and the natural evaporation tended to keep the pits absolutely dry, and the surface layer of fecal matter was quite hard and in spots almost brittle.

Viability of Bacillus typhosus and Bacillus dysenteriæ in Septic Fluids.—The question of the viability of typhoid and dysentery bacilli in septic material bears the same relation to the septic privy that survival in feces does to the pit privy. Consequently an attempt was made to obtain some data bearing on this point. In order to approximate the natural conditions, two privies of the L. R. S. type were constructed and used in the laboratory.

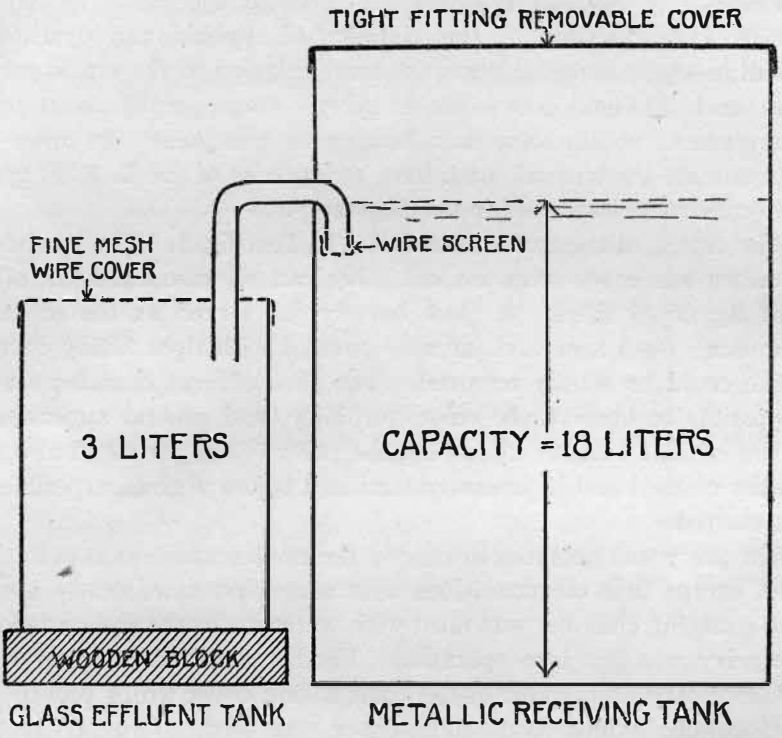
The details of the system are shown in Text-fig. 1. The receiving chamber was made from an old ether can of galvanized tin of a capacity of 25 liters. A glass battery jar served as the effluent chamber. Both tank and jar were covered with tight fitting covers which could be readily removed. The glass effluent chamber made it possible to observe the color, turbidity, and general appearance of the effluent without disturbing the privy. The jar could also be readily cleaned and if necessary sterilized before a given experiment was started.

The privy was operated in exactly the same manner as it is in the field, except that the conditions were somewhat more nearly ideal. The receiving chamber was filled with water up to the siphon before the privy was put into operation. Further addition of water was not necessary on account of the tight fitting cover which prevented evaporation. Only tissue toilet-paper was used. Two privies (A and B) were used by two different individuals on an average of from three to four times a week.

After the tanks had been in operation for about 3 weeks (A from January 3 to 21; B from January 3 to 24), the experiments were started. All experiments were made with stock cultures. A given quantity of a 24 hour broth culture was inoculated into either the septic or effluent chamber and its viability determined by repeated tests for the presence of the inoculated organisms.

Experiment 1.—The effluent from Privy A was inoculated with *B. typhosus* Jan. 22, 1918, and small quantities were removed daily for examination. Up to Jan. 26 the tests were positive (Table IV).

Experiment 2.—This experiment differed from Experiment 1 in that the inoculations were made directly into the septic tank. Tank A received 10 cc. of broth culture of *B. dysenteriæ* Flexner. Tank B received 10 cc. of broth culture of *B. typhosus*. The effluents were then collected at intervals in fresh sterile jars and tested for the respective bacilli. The results are given in Table V.



TEXT-FIG. 1. The Lumsden-Roberts-Stiles privy.

Experiment 3.—The object of this experiment was to determine the effect of temperature on the viability of *B. typhosus* and *B. dysenteriæ*. Partially sterile effluent (filtered through coarse Berkefeld filter) from Tank B was inoculated with broth cultures of *B. typhosus* and *B. dysenteriæ* respectively and divided into three portions. These were kept at 4°C. (ice box temperature), 20°C. (room temperature), and 37°C., respectively, and tested at intervals. The pH of the effluent was 7.4 at the time of inoculation and changed to 8.0 in 24 hours. The results are given in Table VI.

TABLE IV.

Viability of Bacillus typhosus in Septic Effluent.

Culture inoculated.	Date inoculated.	Date tested.	Effluent.	Results.
	1918	*1918*	*pH*	
B. typhosus.	Jan. 22	Jan. 23	8.6–8.8	+
B. "	" 22	" 24	8.6–8.8	+
B. "	" 22	" 25	8.6–8.8	+
B. "	" 22	" 26	8.6–8.8	+ The effluent was removed to a sterile bottle, kept at a temperature of 20–22°C., and the tests continued.
B. "				
B. "				
B. "	Jan. 22	Jan. 28		−
B. "	" 22	Feb. 1		−

TABLE V.

Viability of Typhoid and Dysentery Bacilli in Septic Effluent.

Tank.	Culture inoculated.	Date inoculated.	Date tested.	pH	Results.
A	*B. dysenteriæ.*	Jan. 30	Jan. 31	8.6–8.8	+
	B. "	" 30	Feb. 2	8.6–8.8	+
	B. "	" 30	" 5	8.6–8.8	−
	B. "	" 30	" 8	8.6–8.8	−
B	*B. typhosus.*	" 30	Jan. 31	7.4–7.8	+
	B. "	" 30	Feb. 2	7.4–7.8	+
	B. "	" 30	" 5	7.4–7.8	+
	B. "	" 30	" 8	7.4–7.8	+
	B. "	" 30	" 13	7.4–7.8	+
	B. "	" 30	" 18	7.4–7.8	−
	B. "	" 30	" 21	7.4–7.8	−

TABLE VI.

Effect of Temperature on Viability of Typhoid and Dysentery Bacilli in Septic Effluent.

Culture inoculated.....		*B. typhosus.*			*B. dysenteriæ.*		
Incubation temperature.....		Ice, 4°C.	Room, 20°C.	37°C.	Ice, 4°C.	Room, 20°C.	37°C.
Date inoculated.	Date tested.						
Jan. 24	Jan. 25	+	+	+	+	+	+
" 24	" 26	+	+	−	+	−	−
" 24	" 28	+	+	−	+	−	−
" 24	Feb. 1	+	−	−	+	−	−
" 24	" 4	+	−		−		
" 24	" 11	−			−		

Experiment 4.—The previous experiment showed that temperature was an important factor in determining the survival of typhoid and dysentery bacilli in septic fluid. It was uncertain, however, whether the effect was one of temperature purely or whether the disappearance of the inoculated bacilli was due to overgrowth. The experiment was therefore repeated with sterile effluents. Effluents from Tanks A and B were filtered through Berkefeld candles and the filtrates

TABLE VII.

Viability of Typhoid and Dysentery Bacilli in Sterile Septic Effluents at Different Temperatures.

Date.		Culture inoculated.	Results.			
			Effluent A.		Effluent B.	
Inoculated.	Tested.		Room.	37°C.	Room.	37°C.
Feb. 2	Feb. 4	*B. typhosus.*	+	+	+	+
" 2	" 4	*B. dysenteriæ* Flexner.	+	+	+	+
" 2	" 4	*B.* " Shiga.	+	+	+	+
			Plated 0.1 cc. on Endo plates.			
" 2	" 5	*B. typhosus.*	3,000	Crowded.	Crowded.	Crowded.
" 2	" 5	*B. dysenteriæ* Flexner.	(?)	"	"	"
" 2	" 5	*B.* " Shiga.	1,200	"	"	"
" 2	" 7	*B. typhosus.*	0*	400	400	4,000
" 2	" 7	*B. dysenteriæ* Flexner.	12	12	2,000	4,000
" 2	" 7	*B.* " Shiga.	0	70	40	300
" 2	" 17	*B. typhosus.*	0*	0	Crowded.	Crowded.
" 2	" 17	*B. dysenteriæ* Flexner.	0	0	250	Contaminated.
" 2	" 17	*B.* " Shiga.	0	0	0	0
" 2	Mar. 2	*B. typhosus.*	0*	0	Crowded.	Crowded.
" 2	" 2	*B. dysenteriæ* Flexner.	0	0	—	0
" 2	" 2	*B.* " Shiga.	0	0	—	0

* 0.1 cc. of emulsion diluted to 10 cc. with saline and 0.1 cc. of this suspension spread uniformly over Endo plate.

Effluent A at 20° or 37°C. showed only a slight turbidity after incubation for 24 hrs. Effluent B showed fair turbidity at room temperature and good growth at 37°C. The reaction of Effluent A was: Feb. 4, pH 8.8–9.0; Feb. 7, pH 9.0; the reaction of Effluent B was: Feb. 4, 8.3–8.4; Feb. 7, 8.3–8.4.

tested for sterility. Each filtrate was then divided into three portions and inoculated with broth cultures of *B. typhosus*, *B. dysenteriæ* Flexner, and *B. dysenteriæ* Shiga, respectively. Each of the inoculated lots was then divided into two parts, one of which was kept at room temperature (20–24°C.) and the other at 37°C. Tests were made at intervals for the presence of the inoculated bacteria (Table VII).

TABLE VIII.

Viability of Typhoid and Dysentery Bacilli in Sterile Septic Effluent under Different Conditions.

Inoculated Mar. 5; 0.05 cc. of broth culture to 10 cc. effluent.

Date tested.	Culture inoculated.	Effluent A.*				Effluent B.				Remarks.
		Room.		37°C.		Room.		37°C.		
		Filtered.	Autoclaved.	Filtered.	Autoclaved.	Filtered.	Autoclaved.	Filtered.	Autoclaved.	
Mar. 5	*B. typhosus.*	Crowded.	Crowded.	Crowded.	Crowded.	Crowded.	Crowded.	Crowded.	Crowded.	0.05 cc. plated
" 5	*B. dysenteriæ* Flexner.	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000	direct on
" 5	*B.* " Shiga.	1,200	—	1,200	—	1,000	1,500	1,000	1,500	Endo plates.
" 11	*B. typhosus.*	0	0	0	0	2,000	2,000	2,000	2,000	0.05 cc. spread
" 11	*B. dysenteriæ* Flexner.	0	0	10	0	0	1,000	800	400	on Endo
" 11	*B.* " Shiga.	0	0	0	0	0	0	0	0	plates.
" 13	*B. typhosus.*	0	3	0	0	Crowded.	Crowded.	Crowded.	Crowded.	0.1 cc. plated
" 13	*B. dysenteriæ* Flexner.	2	0	0	0	"	"	"	400	on Endo
" 13	*B.* " Shiga.	0	0	0	0	2	0	0	2	plates.

* The reaction of Effluent A = pH 9.0; Effluent B = pH 8.4.

Experiment 5.—This was a repetition of Experiment 4 and gave essentially the same results. At first there was an increase in the number of organisms, which was followed by a progressive decrease. The experiment was not of any particular value because Effluent B soon became contaminated, and the relative rate of decrease in Effluents A and B could not be followed.

Experiment 6.—This was an elaboration of Experiment 4. The effluents were sterilized by filtration and heat, and the rate of decrease in the number of inoculated bacteria noted (Table VIII).

This experiment again confirmed the results obtained in Experiment 4; namely, that the inoculated organisms died more rapidly in Effluent A than in Effluent B. Since the only obvious and constant difference between the two effluents was their reaction (pH of Effluent A = 8.8–9.0; pH of Effluent B = 8.3–8.4), it would seem that that was the main factor responsible for the destruction of the added bacteria. There is practically no difference in the viability in filtered and heated effluents.

TABLE IX.

Viability of Typhoid and Dysentery Bacilli in Septic Tanks.

Date inoculated.	Date tested.	*B. typhosus.*	*B. dysenteriæ* Flexner.
		Effluent A.*	Effluent B.*
Mar. 5	Mar. 7	+	+
" 5	" 11	−	−
" 5	" 13	−	−
		Effluent B.	Effluent A.
Mar. 23	Mar. 25	+	+
" 23	" 26	+	−
" 23	" 28	−	−
" 23	" 30	−	−

* The reactions of the two effluents were: A = pH 9.0; B = pH 8.4.

Experiments 7 and 8.—These experiments are repetitions of Experiment 3, in order to determine the viability of the test bacteria in the septic tank after it has had a chance to "ripen." The bacteria were introduced into the septic chamber, 5.0 cc. of a 24 hour broth culture being added to each tank. In Experiment 7 the typhoid bacilli were added to Tank A and dysentery bacilli to Tank B; while in Experiment 8 the order was reversed. These experiments again demonstrate the effect of the reaction of the substrate on the viability of the bacteria (Table IX).

Summary of Experiments on the Viability of Typhoid and Dysentery Bacilli in Septic Tank and Effluent.—Experiments 1, 2, 3, 7, and 8 with one exception show that *Bacillus typhosus* cannot be recovered either from the effluent or from the septic tank later than 6 to 8 days after inoculation if kept at a temperature of 20–24°C. The Flexner type of dysentery bacillus was isolated on the 3rd day. In one experiment the typhoid bacilli were recovered on the 14th day after inoculation, and the reaction of this tank during the test period was pH 7.4–7.8. This indicated that the reaction of the fluid was an important factor in determining the viability of test bacilli. Experiments 4, 5, and 6, conducted with sterile effluents having different reactions, tended to confirm this conjecture. In Effluent A, having a pH value of 8.8–9.0, the typhoid bacilli were recovered on the 5th and the 8th days, but not on the 15th and 9th days, respectively. In Effluent B, on the other hand, with a reaction of pH 8.3–8.4, and kept under the same conditions, the typhoid bacilli were recovered on the 30th day. Similar effects were observed in the case of the dysentery bacilli, though the period of survival was shorter. The Shiga variety in one experiment was not recovered on the 5th day in Effluent A at room temperature, but was present in considerable numbers in Effluent B under the same conditions. In the other experiment it was not recovered on the 8th and 9th days in Effluent A but could be isolated on the same days from Effluent B. The Flexner variety behaved in a similar manner, the survival period being 5 and 8 days, respectively, in Effluent A; and 15 and 30 days in Effluent B.

It would appear, then, from these experiments that the Gram-negative bacilli of the typhoid-dysentery group die out rapidly in septic material. The typhoid bacillus may survive for about 5 days, the Flexner type of dysentery about 3 days, while the Shiga bacillus succumbs most rapidly. If the alkalinity of the fluid is low, in other words if the tank is not "ripe," the organisms may survive for a much longer period. The germicidal power of the effluent from a ripe tank is probably due both to the alkaline reaction and to the presence of the antagonistic product of metabolism.

Viability of Typhoid and Dysentery Bacilli in Soil.—What happens to the pathogenic bacilli which survive in the pit material or septic effluent when put on soil? On this question there is an abundance of

literature in fair agreement. However, two important points seem to have been overlooked. Most of the investigators have inoculated soil with pure agar or broth cultures instead of using the fecal or septic mixtures. The two sets of conditions are totally different. Furthermore, while many of the investigators have recognized the effect of moisture, none of them has considered the possible effect of the reaction of the soil.

With a view to determining the relative importance of these factors as influencing survival in soil, the following experiments were made: (1) confirmatory tests to determine the viability of typhoid and dysentery bacilli in soil; (2) the effect of temperature and moisture on viability in the same soil; (3) the relative viability when saline suspensions and fecal emulsions were used as inoculum; and (4) the effect of soil reaction on the viability of these organisms.

Viability of Typhoid and Dysentery Bacilli in Natural Park Soil, Dry and Moist.—The experiments were carried on with park soil taken from the same source. It was not richly cultivated but had

TABLE X.

Relation of Moisture to Survival of Typhoid and Dysentery Bacilli in Soil at Room Temperature.

Series.	Survival of *B. typhosus* in soil.						Survival of *B. dysenteriæ* Flexner in soil.					
	Dry.		Moist.		Wet.		Dry.		Moist.		Wet.	
	days		*days*		*days*		*days*		*days*		*days*	
	+	−	+	−	+	−	+	−	+	−	+	−
1	13	20	26	34	34	39	7	13	39	46	46	56
2	13	20	26	34	26	34	13	20	20	26	46	56
3		25	39	46	26	34	9	14	34	41	34	41
4			32	39	67	74			34	41	73	80

a considerable number of colon bacilli. The soil was dried and coarsely powdered and was then placed in wire baskets which fitted into battery jars, or in glass tubes of 2 inches diameter. The soil was so packed as to furnish different degrees of compactness. To some sets, no water was added; to others, water was added at intervals according to the rate of absorption. In porous soil the water was rapidly absorbed, leaving the soil damp but not wet. In the compact soils there was always some water standing on the surface.

Saline suspensions of 24 hour agar slant cultures were used for inoculation. These inoculations were made on the surface of the soil or in a small hole 1 inch in diameter and $\frac{1}{2}$ inch deep. Samples of the soil were taken either with a large nichrome loop, or, in the later stages, by means of cork borers of different diameters.

The results of this series of experiments are recorded in Table X. The condition of the soil is described by the terms dry, moist, and wet. The survival time is given in days; + indicates the last day isolation was positive; − indicates the day on which isolation was negative.

These experiments confirm those of previous investigators. Moisture is an important factor in the survival of these pathogenic bacilli. In dry soil neither the typhoid nor the dysentery bacilli could be recovered on the 20th day. In moist or wet soil, on the other hand, the bacilli remained alive for 26 to 73 days. There is a considerable variation between the results obtained in the different series, but the viability is consistently greater in soils moist than under dry conditions.

Effect of High and Low Temperature on Viability in Dry and Moist Soils.—Flower pots were used for these experiments. Sixteen pots were filled with the same soil; half of them were left dry and the others

TABLE XI.

Relation of Temperature to the Survival of Typhoid and Dysentery Bacilli in Soil.

B. typhosus.								*B. dysenteriæ.*							
37°C.				4°C.				37°C.				4°C.			
Moist.		Dry.		Moist.		Dry.		Moist.		Dry.		Moist.		Dry.	
days		*days*		*days*		*days*		*days*		*days*		*days*		*days*	
+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−
49	60	*	18	68	75	61	68	*	18	*	18	67	75	25	32

* No test could be made; prevented by mold overgrowth or other reason.

thoroughly moistened. One set of eight pots was inoculated with *Bacillus dysenteriæ* Flexner and another with *Bacillus typhosus*. Pots of each set were kept at 37°C. and ice box temperature (2–4°C.), respectively. In each case one set of pots which was inoculated with typhoid and dysentery bacilli was moistened regularly and kept moist by immersing them in Petri dishes full of water, while the other set

received no water at all. The organisms were inoculated into a shallow hole on the surface of the soil and samples withdrawn at intervals for the isolation of the respective bacilli. The results are summarized in Table XI.

This experiment brings out the fact that typhoid and dysentery bacilli will survive for a longer period at freezing than at body temperature, and again indicates the marked effect of moisture on the viability of these bacteria.

Viability in Fecal Emulsions Placed on Soil.—Suspensions of typhoid and dysentery bacilli were made in saline and fecal emulsions respectively and put on the surface of soil, in the same manner as in previous experiments. The soils were all kept moist and at room temperature. In those experiments in which fecal suspensions were used as the inoculum dysentery and typhoid bacilli could be recovered on the 16th but not on the 20th day; whereas in the soils inoculated with saline suspensions both were recovered on the 35th day, after which time the tests were discontinued.

Effect of the Reaction of the Soil on the Viability of Typhoid and Dysentery Bacilli.—While this work was in progress a number of reports bearing on soil reaction came to my attention. These investigations, notably those by Gillespie (1916), showed that soils varied decidedly in reaction as measured by the hydrogen ion method. Twenty-two loam and clay soils studied by Gillespie showed all gradations from pH 4.5 on the one hand to pH 8.6 on the other. It seemed that this difference in reaction might account for some of the variations in the results obtained by previous investigators as well as in the different series of the present investigation.

To test this possibility, a lot of soil was powdered, sterilized, and divided into three portions. Each portion was adjusted to a different pH by the addition of HCl and subdivided equally among a number of Petri dishes. Two Petri dishes from each of the three lots were inoculated with 1 cc. of broth culture of *B. typhosus*, and similar sets were inoculated with 1 cc. of broth cultures of the Flexner and Shiga varieties of the dysenteric bacilli. The soil and broth were thoroughly mixed with sterile rods to obtain uniform distribution. 1 gm. of soil was then weighed out from each dish for plating, and the rest kept in the dark at room temperature. To maintain constant

TABLE XII.

The Effect of the Hydrogen Ion Concentration of the Soil on the Viability of Typhoid and Dysentery Bacilli.

Lot No.	Initial pH.	pH at end of experiment.	On inoculation.	24 hrs.	72 hrs.	96 hrs.	6 days.	10 days.	30 days.
			No. of *B. typhosus* per gm. of soil.						
1	4.8–5.0	5.4–5.8	40,000,000	1,000,000	400,000	360,000	54,000	8,000	0
2	6.4–6.5	6.4–6.6	40,000,000	13,500,000	12,500,000	12,500,000	14,000,000	24,000,000	540,000
3	7.4–7.6	6.4–6.6	50,000,000,	8,600,000	4,600,000	6,000,000	2,900,000	3,900,000	200,000
			No. of *B. dysenteriæ* Flexner per gm. of soil.						
1	Same as in typhoid		1,000,000	10,000	30,000	14,000	3,000	0	0
2	plates.		5,000,000	7,500,000	4,000,000	5,000,000	7,200,000	4,200,000	40,000
3			5,000,000	11,500,000	9,000,000	3,000,000	2,500,000	2,500,000	380,000
			No. of *B. dysenteriæ* Shiga per gm. of soil.						
1	Same as in typhoid		2,000,000	280,000	300,000	5,000	1,000	100	0
2	plates.		1,000,000	150,000	280,000	150,000	42,000	18,000	9,000
3			5,000,000	8,000,000	3,800,000	3,400,000	1,500,000	2,800,000	80,000

moisture the dishes containing the soil were placed on a stand in a pan of water and covered with a battery jar. The atmosphere in the jar was thus saturated with moisture.

The viability of the bacilli was determined by plating samples of the soil and counting the number of bacteria per gm. The pH was tested by emulsifying 1 gm. of soil in 5 cc. conductivity water, centrifugalizing, and testing the supernatant fluid against colorimetric standards. The results are given in Table XII.

These tests show quite conclusively that the reaction of the soil plays just as important a part in limiting the viability of these bacteria as was the case in the septic tank. In the soil it is the acidity, while in the tanks it is the alkalinity which is destructive to the bacilli.

In the soil having an initial pH of 4.8–5.0 all three organisms suffered a reduction of 90 per cent or over in the first 24 hours, and were practically all dead within 10 days. In the other two soils the mortality rate was less rapid, but all three organisms showed a reduction of 99 per cent in 30 days in the soil with an initial pH of 6.5, and a reduction of over 92 per cent in the same period in the soil having an initial reaction of pH 7.4–7.6. It should be noted that the final reactions of these two soils were identical. It is also noteworthy that these results are in accord with those obtained under other conditions; namely, that the Flexner type of dysentery bacillus reacts more like the typhoid bacillus while the Shiga bacillus is more sensitive to such changes. Variation in the viability of these organisms in different soils is apparently due at least partly to differences in the reaction of those soils.

Other Antagonistic Factors Influencing the Viability of Typhoid and Dysentery Bacilli in Soil.—Martin, in his studies on this question, observed that typhoid bacilli survive longer in sterile than in natural soil. He inferred from these results that the bacteria of the soil exert an antagonistic effect on the typhoid bacillus. This deduction was questioned by Mair, who failed to confirm Martin's results. Frost (1904), on the other hand, working with pure broth cultures of typhoid and soil bacilli showed that *Bacillus fluorescens*, *Bacillus vulgaris*, and *Bacillus vulgatus*, or the products of their growth had a decidedly inhibitive effect on typhoid bacilli.

It seemed of interest to repeat Frost's experiments in order to determine whether the inhibitive effect observed was due to some substance toxic to the typhoid bacillus or whether it was due merely to the unfavorable reaction resulting from the growth of these soil bacteria. Separate cultures of *Bacillus proteus*, *Bacillus fluorescens*, and a spore-forming bacillus isolated from soil were made in Erlenmeyer flasks containing 150 cc. of broth. The inoculated flasks were kept at room temperature for 3 to 4 weeks. The growth was then centrifuged and the supernatant fluid passed through a Berkefeld filter and tested for sterility.

TABLE XIII.

Antagonistic Action of Soil Bacteria on Typhoid and Dysentery Bacilli.

Culture filtrate.	Condition.	No. per cc. on count on inoculation.		Transplants after 48 hrs.		Transplants after 7 days.		Growth in inoculated filtrate as indicated by turbidity.	
		Initial pH 8.4.	Adjusted pH 7.2.	pH 8.4.	pH 7.2.	pH 8.4.	pH 7.2.	pH 8.4.	pH 7.2.
Soil spore.	Heated.	20,000,000	18,000,000	±	+	±	+	+	++
	Unheated.	23,000,000	20,000,000	±	+	±	+	+	++
B. fluorescens.	Heated.	2,500,000,000	12,000,000,000	−	+	−	−	−	−
	Unheated.	2,600,000,000	10,000,000,000	−	±	−	−	−	−
B. proteus.	Heated.	6,000,000,000	8,000,000,000	−	+	−	+	∓	++
	Unheated.	10,000,000,000	8,000,000,000	−	+	−	+	∓	+

The + and − signs indicate the relative abundance of growth. ++ = turbidity equal to typhoid broth culture; ∓ very scant growth; − no growth; + and ± amounts intermediate between ++ and ∓.

The sterile filtrates were then treated as follows: Each filtrate was divided into two parts, one of which was kept in a boiling water bath for ½ hour. The heated and unheated lots were then each subdivided into two portions, one of which was left unaltered, while the other was adjusted to a reaction of pH 7.2–7.4. The four lots were then tubed with sterile precautions, 10 cc. to a tube. Tubes from each lot were inoculated with 0.1 cc. of broth culture of typhoid or dysentery bacilli. Plates were made immediately to determine the relative number of organisms per cc. On subsequent days subcultures were

made with a standard 4 min. loop to agar slants. The striking results which were obtained are recorded in Table XIII.

Exactly the same results were obtained with *Bacillus dysenteriæ* Flexner. Filtrates from one other strain of *Bacillus fluorescens* and *Bacillus proteus* yielded similar results to those given above.

It would seem, therefore, on the basis of these experiments, that *Bacillus fluorescens* is the only organism of those studied which actually liberates some substance antagonistic to the growth of the typhoid and dysentery bacilli. In the filtrate from the proteus cultures improvement in the growth was obtained in the adjusted tubes by heating, but the most marked inhibitive effect was evidently due to the reaction of the filtrate. The filtrate of the culture of the spore former was the least inhibitive of those tested although its reaction was the same as that of the other filtrates.

TABLE XIV.

Antagonistic Action of Soil Spore Former on Typhoid Bacilli.

Date.	No. of typhoid bacilli per cc. of broth on brilliant green plates.
Inoculated Mar. 26.	100,000
Mar. 30	Too crowded.
Apr. 1	9,000,000,000
" 6	10,000,000,000

The toxic effect of brilliant green on spore-bearing bacilli made it possible to test directly the antagonistic action of this organism on the typhoid bacillus. Both cultures were inoculated into broth and incubated in the usual manner. Counts were made on brilliant green plates by spreading a given volume of culture on the plate and incubating. The results showed that the two bacteria grew equally well together (Table XIV).

Summary of the Experiments Bearing on the Survival of Typhoid and Dysentery Bacteria in Soil.—These experiments confirmed on the whole the results obtained by previous investigators; namely, that typhoid and dysentery bacilli may survive in soil for varying lengths of time depending on moisture. In moist soil the bacilli could be recovered up to the 70th day but were not recovered on the 80th day after inoculation. In dry soil they died out more rapidly, their viability in no case extending up to the 20th day.

In addition to confirming previous observations these experiments brought to light some new and interesting facts. It was shown quite conclusively that the reaction of the soil as well as moisture, greatly affected the viability of the bacteria in question. In soil having an unfavorable reaction even though favorable moisture conditions, 90 per cent of the introduced bacilli died in the first 24 hours, 99 per cent in 6 days, and practically all of them about the 10th day. When the soil reaction was favorable (pH 6.4–7.5), the reduction in numbers was slow, but over 90 per cent of the bacteria died out within 30 days.

When typhoid and dysentery bacilli were added to soil in fecal suspensions, the length of time after which they could be recovered was not so great as when saline suspensions were used. As it was not possible to ascertain whether this variation signified actual death of the pathogenic bacilli or merely failure to isolate them on account of overgrowth, the experiments were not extended further.

An examination of the nature of the antagonistic action of soil bacteria in broth cultures on the typhoid and dysentery bacilli confirmed in some respects the work of Frost, but indicated that at least two elements were concerned in this process. One important element is the alkaline end-reaction of broth cultures of these bacteria which is unfavorable to the initiation of growth and favorable to the disintegration of the bacterial cell. *Bacillus fluorescens* and to a lesser extent *Bacillus proteus* apparently exert an inhibitive effect distinct from that due to the reaction. The inhibitive substance of *Bacillus fluorescens* is not entirely heat labile, while that of *Bacillus proteus* cultures seems to be completely destroyed by heat.

It has not been determined whether or not these organisms exert the same antagonistic action in soil as they do in broth, but it is conceivable that under certain conditions they would have that effect.

It would seem, then, that the speed with which typhoid and dysentery bacilli die off in soil would depend on a number of factors. Chief among these are the moisture content and reaction of the soil. The nature and abundance of other flora might play a secondary part. The important fact is that in dry or acid (moist or dry) soils most of the pathogenic bacteria would die off within 10 days.

Penetration of Bacteria through Soil.—The question of the power of bacteria to penetrate through soil is exceedingly important in its

bearing on the problem of soil pollution. The possibility of the passage of pollution through cracks in the soil or subsoil is not open to question. It is doubtful, however, whether bacteria can penetrate through layers of soil, and the available data on this subject do not furnish a conclusive answer.

Experiments have been made, therefore, in order to throw some light on this obscure point. The purpose of these experiments was to determine, (1) whether typhoid and dysentery bacilli were capable in themselves of spreading through soil in different directions and (2) whether they could be so distributed mechanically by water and if so to what extent.

To determine what might be termed the natural passage of typhoid and dysentery bacilli through soil the following methods were used.

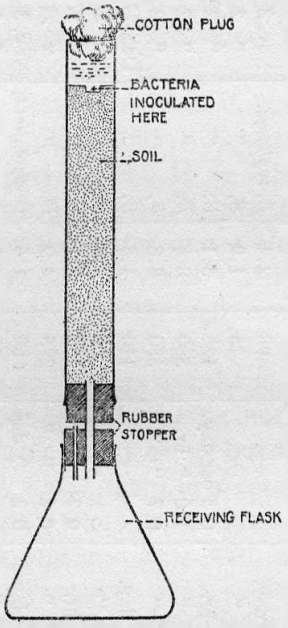
Round wire baskets of ½ inch mesh, 12 inches long, and 6 inches in diameter, were filled with fresh finely divided park soil. By adding the soil slowly and shaking it down at frequent intervals it was possible to render it fairly compact. It was then thoroughly moistened and allowed to set for 2 to 3 days, after which the basket was placed in a battery jar covered with wire gauze to keep out insects. A small hole of about 1 cubic inch was then made on top of the soil, at the center, into which the bacterial suspension was placed. By means of an ordinary cork borer small enough to fit into the holes of the wire basket, soil samples were taken, at intervals for a period of a month, from different depths and at various distances from the point where the bacteria were placed. It should be added that the soil was kept moist by the addition of water near the edge of the basket in order to disturb the bacteria as little as possible.

Twelve of these experiments were made, six with typhoid and six with dysentery bacilli. At no time could either of these organisms be recovered from soil at more than 1 inch away from the point of inoculation.

The experiment was then varied by putting the soil in flower pots 8 inches by 8 inches. The inoculation was made as before, but the moisture was kept rather constant by keeping the pots in dishes filled with water. Samples of soil taken 3 inches away from the point of inoculation at no time contained the inoculated bacilli. Tests of the water in the dish as well as of the soil samples taken from the hole at the bottom of the pots were all negative.

The experiments with regard to the mechanical distribution by water were different in character from those just described. For this

purpose glass tubing about 1 inch in diameter was used. Tubes of different lengths were cut and one end was closed with a one-hole rubber stopper into which was fitted a bit of glass tubing flush with the inner end of the stopper and projecting for about an inch at the outer end. A piece of gauze was fitted over the inner end of the stopper to prevent clogging of the hole and the tube filled with soil. By adding small amounts of finely divided soil and shaking it down each time, one could easily obtain a uniform column of soil. By shaking with various degrees of frequency and vigor it was possible to obtain columns of soil of different degrees of compactness.



TEXT-FIG. 2. Apparatus for determining penetration of bacteria through soil.

The soil was then thoroughly moistened and allowed to set. The relative compactness of each column was measured roughly by determining (1) the absorption time of a given volume of water added at the top of the soil column, and (2) the speed with which water added at the top of the column began to appear at the other end, or, in other words, the rate of passage through the soil. The outfit used in this experiment is shown in Text-fig. 2.

TABLE XV.

Penetration of Typhoid and Dysentery Bacilli through Soil.

Tube No.	Length of tube.	Rate of absorption per sq. in.	Rate of passage through soil.	Total amount of water added.	Average amount of water added daily.	Culture inoculated.	Date inoculated.	Test bacteria in effluent.
	in.	*cc.*	*in.*	*cc.*	*cc.*		*1917*	
1	9	2.0 per day.	—	95	2.4	*B. typhosus.*	Nov. 2	Water did not pass through in 40 days.
2	30	2.0 " "	—	75	1.9	*B.* "	" 2	
3	70	1.0 " hr.	0.5 per day.	125	3.1	*B.* "	" 2	— Dec. 1, Dec. 7, and Dec. 10.
4	18	6.0 " "	1.0 " hr.	350	8.8	*B.* "	" 2	+ Nov. 27 and Dec. 4, — after that.
5	36	5.5 " "	0.7 " "	350	8.8	*B.* "	" 2	—
6	13	2.0 " day.	—	110	1.8	*B.* "	Dec. 27	Effluent —.
7	25	2.0 " "	—	50	0.8	*B.* "	" 27	" —.
8	16	0.13 " hr.	0.21 per hr.	130	2.1	*B.* "	" 27	" —.
9	22	0.45 " "	0.8 " "	295	4.9	*B.* "	" 27	+ Feb. 7, — after that.
10	20	1.0 " day.	0.2 " day.	60	1.5	*B. dysenteriæ.*	Nov. 3	—
11	12	2.0 " "	—	90	2.3	*B.* "	" 3	—
12	30	Extremely slow.	—	100	2.5	*B.* "	" 3	—
13	24	6.0 per hr.	0.5 per hr.	320	8.0	*B.* "	" 3	+ Nov. 23, — after that.
14	20	—	—	40	0.7	*B.* "	Dec. 27	—
15	22	0.25 per hr.	0.4 per hr.	580	9.7	*B.* "	" 27	—
16	29	—	—	50	0.8	*B.* "	" 27	No effluent.
17	70	—	—	50	0.8	*B.* "	" 27	Effluent — Jan. 28, 1918.

By varying the length of tube and the degree of compactness it was possible to measure the effect of these two factors on the penetration of the bacteria.

The test cultures were put into a small central hole at the top of the column. Water was added regularly in varying amounts in order always to keep a constant column of water (about 2 inches) on the surface of the soil. The less compact tubes thus naturally received a great deal more water than the more compact ones. The water that filtered through the soil was caught in small sterile Erlenmeyer flasks and examined for the presence of the inoculated bacilli. The experiments were always continued for some time after the test organisms could no longer be recovered from the point where inoculated. The results of these experiments are shown in Table XV.

The results of these tests show that in course of time, that is, in about 20 or 25 days, typhoid and dysentery bacilli may be carried through a column of pervious soil $1\frac{1}{2}$ to 2 feet deep. The bacteria were not carried through 3 feet of soil of similar character. In more compact soils in which the water passes through more slowly, the inoculated bacteria were not recovered. The fact that it takes about 20 days for the bacteria to come through about 2 feet of soil indicates that the passage is a mechanical one and that it is dependent on the permeability as well as on the depth of the soil.

The conditions of the experiment do not correspond with those occurring naturally during heavy rains; but they are similar to those usually found in a pit where there is a certain amount of stagnant water. As a rule, pits are more or less protected against direct rain, and although they may fill up partly with water in the course of a rain the material in them is not as a rule subjected to the driving force of the rain. The results of the experiments may, therefore, bear on the possible transportation of pathogenic bacteria through the soil by the stagnant water.

Summary of Experiments on Penetration.

The results of the various tests of the penetrability of bacteria through soil show that the introduced pathogenic bacteria do not of themselves spread laterally or in any other direction. These results

corroborate the findings of Abba and his coworkers, Grancher and Deschamps, Robertson, and others. The other fact brought out by these experiments is that in certain pervious soils water may transport these bacteria through a depth of at least 2 feet, but that the passage takes some time, under the conditions of the experiment at least 3 weeks. In compact soil, however, the bacteria do not appear to be carried through even 1 foot of soil.

GENERAL SUMMARY OF THE LABORATORY INVESTIGATIONS ON VIABILITY AND PENETRABILITY THROUGH SOIL OF TYPHOID AND DYSENTERY BACILLI.

The experiments detailed above are not exhaustive and do not warrant any sweeping deductions. It seems convenient at this point, however, to summarize the main facts as observed in order to discover their possible interrelation.

1. In *feces* the typhoid bacilli were recoverable up to the 10th day and not thereafter; the dysentery bacilli up to the 8th day. The survival period is shorter in loose than in solid feces. These results are in accord with those reported by Gärtner, Park, and Kruse.

2. In *pit* material kept dry by the addition of powdered soil, the typhoid and dysentery bacilli were not recovered on the 10th and 4th days respectively, while the paratyphoid B bacillus was recovered on the 10th, but not on the 15th day. These results differ from those obtained by Galvagno and Calderini who recovered typhoid bacilli after 15 to 30 days, but these authors experimented with ordinary pits without the addition of soil, and the difference in moisture may account for the differences in results.

3. In *septic* fluids the reaction is the important factor in controlling the viability of typhoid and dysentery bacilli. When the reaction was pH 7.4–7.8 the typhoid bacilli could be recovered on the 14th day, and the dysentery bacilli on the 8th day. When the reaction was pH 8.6 or over, the bacilli died out more rapidly and were recovered only on the 5th and 3rd day, respectively.

4. In *soil* the typhoid and dysentery bacilli behaved alike. The main factors influencing their viability were the moisture content and the reaction of the soil. In moist soil the inoculated bacteria might survive for 70 days, although the greater number (about 90

per cent) of them died within 30 days. In dry soil the bacteria were not recovered on the 20th day. In acid soil (pH 5.0–5.5) the typhoid and dysentery bacilli were almost all dead on the 10th day, irrespective of the moisture. These results are in general accord with those of previous investigators (Dempster, Pfuhl, Firth and Horrocks, Mair), and also explain the variations in the figures obtained by various authors. Part of the antagonistic action of the soil may be due to inhibitive substances produced by other bacteria. Such substances have been found in broth cultures of *Bacillus fluorescens* and *Bacillus proteus*. But in the main the reaction of the soil and the amount of moisture are the significant elements.

5. The typhoid and dysentery bacilli do not spread either laterally or otherwise through the soil unless carried mechanically by water. The direction of penetration is in the direction of the flow of the water. In relatively porous soil the bacteria were carried through a depth of 2, but not 3 feet. In compact soil the bacteria were not carried through 9 inches of soil. Although these experiments were somewhat different in character from those reported by Firth and Horrocks and by Robertson, the findings are similar to theirs.

FIELD INVESTIGATIONS.

Studies of the Pit Privy.—Since the most important element of danger in the pit privy is the possibility of subsoil and ground-water pollution, attention has been focused on that question. There were two methods available for the attack on this problem: (1) To sink wells to different depths and at different distances from the pit, collect the ground-water, and test it for pollution; and (2) to collect specimens of soil at different depths and distances from the pit and examine them for pollution. The first procedure implies the intensive study of a few pits over a long period, is subject to variation with the fluctuation in rainfall, and involves the likelihood of contamination with surface wash. The second method, on the other hand, permits of an extensive survey of a large number of closets in different kinds of soils, both before and after rainfall; and also makes it possible to trace the direction of the seepage, if any occurs. In this investigation the second method was used exclusively.

Collection of Samples.

Soil Specimens.—The soil samples were taken with an auger having a protecting sleeve over the boring end and fitted with adjustable rods. A hole $1\frac{1}{2}$ feet deep was first made with a small auger, the boring diameter of which was $2\frac{1}{2}$ inches. A protecting plate with a tube about 8 inches long was then placed in the hole. The sampling auger which had a boring diameter of $1\frac{1}{2}$ inches, moved loosely in the protecting tube, and was thus prevented from contact with the upper layer of soil. Specimens were taken at two or three depths in the same hole. Usually three or four holes were sounded for each privy at distances roughly of 1, 3, 5, and 10 feet, respectively. Just before the sample was taken the auger was cleansed thoroughly with a 5 per cent lysol solution and dried with a sterile cloth. The soil was removed from the auger with a sterile glass rod into a sterile wide-mouth, glass-stoppered bottle and covered with tin-foil.

Water Samples.—The water samples were taken in the usual way. In open bucket wells, the bucket in the well was lifted and the water poured into the bottle. The contamination contributed by the bucket is thus represented in the results.

Transmission of Samples.—In the early part of the work the samples were brought to the laboratory the same day and tested immediately. Later that was not feasible and the samples were shipped in ice containers and usually examined within 24 hours after the time of collection. Special tests on a number of samples showed that little or no change takes place in the soil samples in 48 hours, if they are kept moderately cool (12–15°C.).

Testing of Samples.—The soil samples were removed to a sterile Petri dish or piece of paper and thoroughly mixed with a sterile glass rod. Portions weighing 10 to 25 gm. were then weighed, placed into wide-mouth, glass-stoppered bottles, and sterile water was added in the ratio of 2 cc. to 1 gm. of soil.

After thorough shaking, the bottle was allowed to stand until the heavy particles settled. The supernatant suspension was then inoculated into lactose broth and lactose bile. At first, equivalents of 1.0, 0.1, and 0.01 gm. were inoculated; later 5.0, 1.0, and 0.1 gm. portions were used. At the same time 0.1 cc. of the suspension was

spread on an Endo and 0.2 cc. on a brilliant green plate. The tubes and plates were then incubated and examined after 24 and 48 hours, respectively. All tubes that showed gas were streaked on Endo plates to confirm the presence of *Bacillus coli* and also to catch pathogenic as well as any other non-lactose-fermenting types of bacilli that might be present. In addition to this all the bile tubes containing the highest concentration of soil suspension, that showed gas were held for 48 to 72 hours and streaked on Endo and brilliant green plates. This was done because it was found that the bile exerts a marked inhibitory effect on the soil bacteria as well as on anaerobic spore formers but permits the members of the typhoid and dysentery group of bacteria to grow. It seemed likely, therefore, that if any typhoid or dysentery-like bacilli were present they would be numerous enough on the 2nd or 3rd day to appear on the plate. In addition to fishing suspicious colonies from these and the direct plates, typical colon-like colonies were picked from each plate and put through a number of confirmatory tests,—sugar fermentation, indole production, and methyl red reaction. The purpose of this was twofold; (1) to confirm the presence of *coli*, and (2) to differentiate the *Bacillus coli* from the *Bacillus aerogenes*.

The water samples were tested in the same manner, 10, 1.0, and 0.1 cc. portions being used for inoculation.

It was hoped that by the aid of these somewhat elaborate tests it would be possible not only to detect pollution but also to isolate specific pathogenic bacilli, if present.

Material for Study.—In order to study conditions as they exist, headquarters were established at Columbia, S. C.,[2] and a number of communities located in different sections of the state and representing different soil conditions were chosen for study. Each of the communities selected had been previously worked in by a representative of the State Department of Health. In practically all of them the pit privy had been installed under the supervision of the agent. It was thus possible to know exactly the length of time a pit had been in use.

[2] We are indebted to Doctor Hayne of the State Board of Health for the privilege of the use of the Department Laboratory.

The communities selected were: *Reidville*, Spartanburg County; in the Piedmont region, largely a hard, compact red clay soil. The pits were installed 3 years before the time the tests were made. *Bethel*, Sumter County, and *Sardis*, Florence County; both in sand-clay regions, with pits constructed in August and March, 1916, respectively, or 9 months to 1 year previous to the time of testing. *Luray*, Hampton County; sandy soil on a sandstone bed with a water-table 8 to 10 feet below the surface. The pits were completed in November, 1915, or about 18 months before the tests were made. *Kitchings Mill*, Aiken County; sandy and sand-clay soil, with pits dug 2 years prior to the test.

Consequently the pits studied had been in use anywhere from 1 to 3 years, represented practically all the soil conditions in the state, and were probably typical of those under similar soil conditions in other states.

The results obtained in this investigation may best be presented under a number of subheadings.

Surface Soil and Subsoil Pollution.

It is obvious that before attacking the problem as a whole it was necessary to ascertain the normal condition of the soil. A number of observers (Houston, Chick, and others) have found *Bacillus coli* in soil, particularly in cultivated soil. Should it be found that the

TABLE XVI.

Surface Soil and Subsoil Pollution.

	Depth in ft. below surface.				
	0–1	2–4	4–6	6–8	8–10
Total No. of samples	12	5	14	5	9
No. of samples polluted	11	0	0	0	0

organism is likewise present in the lower layers in the subsoil, the attack on the problem from this angle would be, to say the least, difficult.

A series of preliminary tests of the surface soil and subsoil was therefore made, with encouraging results. The surface soil was invariably

found to be grossly contaminated. The subsoil, on the other hand, was entirely free from pollution. Of twelve samples of soil taken within less than 1 foot from the surface, eleven were contaminated with *Bacillus coli*. Of thirty-three subsoil samples taken 2 to 10 feet below the surface not one was found to be contaminated. The results are given in Table XVI.

Subsoil Penetration from Pits and "Septic Pits."

With the subsoil, as a rule free from pollution, the soil sample method could readily be applied to the study of seepage from pits or other privies. Owing to the fact that the pit was the type of privy prevalent in these communities, attention was devoted mainly to the study of this type. In the course of the work, however, two examples of an interesting device were encountered and studied. This type will be designated for convenience the "septic pit" and will be discussed more fully.

Seepage from Pits.—The line of seepage from pits was followed by taking samples at varying depths and at different distances from the pit. The general direction of flow or penetration is shown in Table XVII.

These results (Table XVII) are typical during dry weather. There is no evidence of lateral seepage. All the samples taken above the bottom of the pit even if only 1 foot away were negative. There is, however, clear indication of vertical penetration for a depth of 2 to $3\frac{1}{2}$ feet below the pit. There are also definite indications of lateral extension below the base of the pit for a distance of at least 2 to 3 feet. Beyond these indicated depths and distances there was no evidence of pollution.

A better idea of the relation of pits to soil pollution may be obtained from the summary given in Table XVIII, which shows the total number of samples at the different distances and depths tested, and the relative number found polluted. In the main it brings out more strikingly the points indicated in the individual protocols.

Table XIX contains data given in Table XVIII correlated with the type of soil. It shows on the whole that the extension of pollution is approximately the same in all the soils studied.

TABLE XVII.

Penetration of Pollution from Pits into the Surrounding Soil.

Depth of pit.	Depth of sample.	Distance from pit.	Results: *B. coli*.	Type of soil.	Age of pit.	Remarks.
ft.	*ft.*	*ft.*			*yrs.*	
5	5½	1	+ 0.1 gm.	Clay.	3	Reidville. Ground-water 20 to 25 ft. below surface.
	6	3	−			
	8	3	−			
3½	3	1	−	Sand, sand-clay.	1	Sardis.
	5	1	+ 0.1 gm.	Water.		
	3	2	−	Sand.		
	5	2	+ 0.01 gm.	Water-paste.	1	Water was struck at 5 to 6 ft. below the surface.
	3	6	−	Sand.		
	4½	6	−	Water-paste.		
	4½	15	−	Sand-clay.		
	6	15	−	Cement-like.		
3	3	1	−	Sand-clay.	2	Kitchings Mill. Hard dry soil difficult to bore.
	5	1	+			
	6	1	−			
	5	3	+			
	6	3	−			
	6	5	−			
3	3	1	+	Clay.	1	Bethel.
	4½	1	−			
	3	3	−			
	6	3	−			
	4	10	−			
	6	10	−			
4	4	1	−	Sandy soil.	1	Kitchings Mill. Water struck at 8 to 10 ft. from top.
	6½	1	+	Moist, paste-like.		
	8	1	−			
	4	4	−			
	7	4	−			
	9	4	−	Moist, paste-like.		
	5	10	−			
	9	10	−	Water.		
3	3½	1	+	Sand.	1	Bethel.
	6½	1	+	Clay.		
	9	1	−			
	4	1½	−			
	6	1½	+			
	5	2	−			
	10	2	−			
	4	5	−			
	7	5	−			

TABLE XVIII.

Presence of Pollution at Different Distances from Pits and at Various Depths from the Surface.

Depth from surface.	Distance from pit in feet.					
	1–3		4–6		7–15	
	Total.	Polluted.	Total.	Polluted.	Total.	Polluted.
ft.						
2–4	11	5	3	0	2	0
4–6	16	7	8	0	6	0
6–8	7	3	2	0	3	0
8–10	8	1	7	0	2	0
Total.....	42	16	20	0	13	0

TABLE XIX.

Relation of Type of Soil to Extension of Pollution from Pits.

No. of pits.	Type of soil.	Distance of pits in feet.					
		1–3		4–6		7–15	
		Total.	Polluted.	Total.	Polluted.	Total.	Polluted.
7	Sand-clay.	25	11	11	0	11	0
2	Sand.	8	1	5	0	2	0
2	Clay.	6	1	0	0	0	0

Seepage from Septic Pits.

The name "septic pit" is applied to a curious closet two examples of which were found in one community. They are leaching cesspools without a brick lining and are covered over with a board roof and a foot or more of soil. Unlike the cesspool, they had an outlet as well as an inlet pipe, both near the top of the hole, the latter connected with the water-closet in the house and the former carrying the overflow to a subsoil tile pipe. Being dug in hard clay, they were always full of water and consequently were really crude septic tanks in which the solid excrement settled to the bottom and was partially liquefied, while the fluid portion overflowed whenever the hole filled up to the level of the outflow pipe.

Both holes were in dense clay soil and had been in use for over 2 years, thus offering an excellent opportunity for studying subsoil penetration of contaminating organisms. Both were full at the time the tests were made. One (A) was 16 feet long, 12 feet wide, and 6 feet deep. The other (B) was of the same length and width but was 7½ feet deep. Pit A was located on the down slope from the

TABLE XX.

Seepage into Soil from Septic Pits.

No.	Depth.	Depth of sample.	Distance from pit.	Results.	Remarks.
	ft.	*ft.*	*ft.*		
A	6	4	1	+	
		5	5	+	
		7	5	−	
		5	5	−	
		7	5	−	
		6½	2	−	
		9½	2	−	
		6	6	−	
		8	6	−	
B	7½	5½	1½	+	Caved in at 6½ ft.
		4½	5	−	
		6	5	+	Caved in at 7 ft.
		6	5	+	Another hole.
		7½	5	+	Water and sandstone 5–7 ft.
		5	8	−	
		6	12	+	
		6	18	+	Sandstone and water.
		6	30	−	Clay.
		8	30	−	Clay.
		10	30	−	Clay.

house about 150 feet from the well in solid clay. Pit B was similarly located except that at its base was a vein of sandstone forming a water-table at a depth of about 8 to 10 feet from the surface.

Soil samples were taken around these pits and tested in the manner previously outlined. The results are especially interesting because they indicate rather strikingly the real source of danger that must be guarded against in recommending the use of pits. Pit A was located

in hard clay soil with the water-table about 30 feet below the surface. As was the case in the dry pits, the only pollution that could be detected was that due to soakage. Owing, no doubt, to its long continued use and the large amount of water always present in the hole, the pollution was found at points 5 feet away from the pit, whereas in the dry pits it extended as a rule only 3 feet. Pit B, on the other hand, although in the same type of hard clay soil, was immediately above a sandstone ledge which was only 3 to 8 feet below the surface and which created a water-table at that level. This water-table, although apparently static, was evidently connected with the drilled pump and contributed a constant stream of pollution to the well. By actual soil tests it was possible to trace the pollution a distance of 18 feet from the pit. Whenever the stratum of sandstone was struck in the boring, the soil sample was found to be polluted; the course of the seepage was unmistakable.

The results of the tests on these septic pits are summarized in Table XX.

Relation of Season to Soil Pollution.

The results just reported were obtained during the months of April, May, and June. A more extensive series of pits was studied in the latter part of June and during all of July. The spring was unusually cool and practically without rain. July, however, was very hot and was characterized throughout by excessive precipitation. The results of the tests made toward the end of June were in general agreement with the earlier ones, but the July results differed in many respects. Pollution extended deeper into the soil and further from the pits than in the previous tests, particularly in sandy soils.

Altogether, 43 pits were studied in this series, a total of over 260 samples of soil having been examined. Table XXI is a summary of the results obtained in the different soils, showing the relative pollution at various depths and distances. It will readily be seen from the table that the subsoil was generally more widely contaminated than in the earlier tests, and also that of the three types of soils studied the clay soil shows a relatively smaller number of polluted samples than either of the other two, and the sandy soil the greater number.

It is significant that a larger proportion of the samples taken at a distance of 5 feet or more from the pits than of those taken at 3 to 4 feet were polluted.

TABLE XXI.

Pollution of Different Types of Soils at Various Depths and Distances from the Pit.

Depth from surface.		Distance from pit.															Total.			Per cent polluted.		
		1–2 feet.			3–4 feet.			5–7 feet.			7–15 feet.			15–30 feet.								
		S	SC	C	S	SC	C	S	SC	C	S	SC	C	S	SC	C	S	SC	C	S	SC	C
ft.																						
1–3	Total.	5	2	4		1	2										5	3	6	60	33	16
	Polluted.	3	1	1		0	0															
4–6	Total.	28	28	17	11	9	12	10	13	10	7	1					57	51	39	65	39	36
	Polluted.	19	11	8	9	4	4	6	4	2	3	1										
7–10	Total.	7	10	10	7	7	8	13	13	3	8	7	2	3	3	1	38	40	24	52	42	21
	Polluted.	3	3	1	3	2	1	9	4	1	4	5	2	1	3	0						
Total.		40	40	31	18	17	22	23	26	13	15	8	2				100	94	69	60	40	29
Polluted.		25	15	10	6	6	5	15	8	3	7	6	2									
Per cent.		62½	37½	31	33	35	23	65	30	23	47	75	100									

S indicates sand; SC, sand-clay; and C, clay.

TABLE XXII.

Relative Number of Pits in Different Soils near Which Pollution Was Detected.

	Total.	Sand.	Sand-clay.	Clay.
No. studied	43	17	15	11
Pollution absent at 5 ft	23	6	9	8
" " " 10 ft	2	2	0	0
" present " 5 ft. or more from pit	18	9	6	3
Positive, *per cent*		53	40	27

The difference in the degree of pollution of the three soil types studied is further emphasized when the results are tabulated on the basis of the number of pits studied and the number showing pollution at distances of 5 feet or more. In the clay soil, contamination was found at a distance of more than 5 feet in the case of three out of eleven pits studied, that is in 27 per cent; in the sand-clay soil in six out of fifteen pits, or 40 per cent; and in the sandy soil in nine out of seventeen pits, or 53 per cent (Table XXII).

An explanation of the difference observed in the three soils, particularly between the clay soil and the others, is suggested by a comparison of the dates at which the tests were made (Table XXIII).

It is clear that the soils studied after July 1 showed a greater degree of contamination. The rainy season began early in July, the first heavy rain recorded being on July 4. This rather striking correlation supports the view that the greater extension of pollution in this series is due to the heavy rains.

TABLE XXIII.

Relation of Season to Difference in Pollution in Clay, Sand-Clay, and Sandy Soils.

	Type of soil.		
	Clay.	Sand-clay	Sand.
No. of pits studied before July 1*	9	3	0
" " " " after July 1	2	12	17

* Rains started at this time.

Source of Increased Pollution.—Even if it is accepted that the rain was accountable for the increased contamination of the soil, the question still remains: Where did the pollution come from? It may either have spread from the pit, or have been washed down from the surface, or it might have been derived from both sources. The results obtained by Robertson and by Firth and Horrocks with *Bacillus typhosus*, those by Davies and Tyndale with sewage, and the experimental and earlier field tests in this investigation, showed clearly that there is ordinarily no lateral seepage through soil. There still remains, however, the possibility of penetration when the soil is deluged by a succession of heavy rains, although it is extremely unlikely that the extension would be lateral.

There are a number of indications in these tests which point to the surface as the probable source of pollution. The tests made in connection with the septic pits showed that there was little or no lateral diffusion even though these holes were almost always partly or completely filled with water for a considerable length of time. Attention is also called to the fact that proportionately more samples were polluted at distances of 5 feet or over than at 3 to 4 feet. Furthermore, the proportionately larger number of *Bacillus aerogenes* isolated

from the second series of samples is suggestive, in view of the recent reports by Johnson and Levine, Burton and Rettger, Winslow and Cohen, and others concerning the prevalence of this type in soil.

TABLE XXIV.

Pollution of Subsoil at Different Distances from the Pit.

Depth of pit.	Distance from pit.	Depth of sample.	Results.	Type of soil.
ft.	*ft.*	*ft.*		
4	1	5	+	Sand.
	1	7½	−	
	3	5	−	
	3	7	−	
	5	7	+	
	1	6	+	Sand.
	3	6	−	
	3	8	−	
	5	7	+	
	5	9	−	
	3	3½	−	Sand.
	3	5½	−	
	5	4½	+	
	5	7½	+	
	10	5½	−	
	10	7½	−	
	2	5	+	Sand.
	2	6½	−	
	5	5½	+	
	5	7½	+	
	10	6	−	
	1	6	−	Sand-clay.
	1	7½	−	
	3	6	−	
	3	8	+	
	5	8	+	

Of the 104 strains obtained from the first series, 18, or 17 per cent, were *Bacillus aerogenes;* while of the 145 strains from the second series 63, or 44 per cent, belonged to that type. These strains were obtained from all the samples, those near the pit as well as those distant

from it. The presence of *Bacillus aerogenes* in so many of the polluted samples of the second series would indicate surface contamination, since it is now recognized that this organism is more indicative of soil than of fecal pollution.

A further suggestive indication is the fact that in a number of instances the subsoil near the pit was not polluted, while that at a distance from the pit was contaminated. This was indicated in a general way in Table XXII, but is brought out more strikingly in Table XXIV.

Although all the results cited create a strong probability that the surface soil was the source of the pollution, they do not definitely prove that that is actually the case. There still remains the other possibility; namely, that heavy rains do tend to carry pollution to greater distances from the pit.

Penetration of Bacteria from the Surface.

If it is assumed that the subsoil is contaminated from the surface, it must be shown that the rain is capable of carrying bacteria to a depth of 10 feet. Firth and Horrocks were unable to wash *Bacillus typhosus* through 2 feet of compact soil with a rain of 3.5 inches per hour falling for 5 hours. Grancher and Deschamps were unable to wash *Bacillus typhosus* through 8 feet of soil. Abba and his coworkers, on the other hand, showed that *Bacillus prodigiosus* could be washed through 2 to 3 meters of soil. In our own experiments it was shown that water could carry bacteria through 2 feet of soil but not through 3 feet. These results were obtained with stagnant water working its way down through a column of soil. It is entirely probable that the force of heavy rains would carry bacteria through a much greater depth in a shorter time, especially in porous sandy or sand-clay soils.

A few direct observations made in the field bear on this question and indicate the extent to which the penetrative force of dropping water may carry bacteria. A driven pump 14 to 15 feet deep was found to be grossly polluted. Repeated tests showed the pollution to be constant, although it varied in amount. Samples were taken of the soil between the pit and the well, but the pollution could not be traced to the pit or other source. It was noted that one spot near

the mouth of the pump constantly receiving the pump drippings and overflow, was thoroughly soaked. It seemed possible that the drippings and waste water slowly found their way into the well and carried the contaminating organisms with them. Samples were taken at different depths on the wet and dry side of the pump, with the results shown in Table XXV.

TABLE XXV.

Pollution of Subsoil by Pump Drippings.

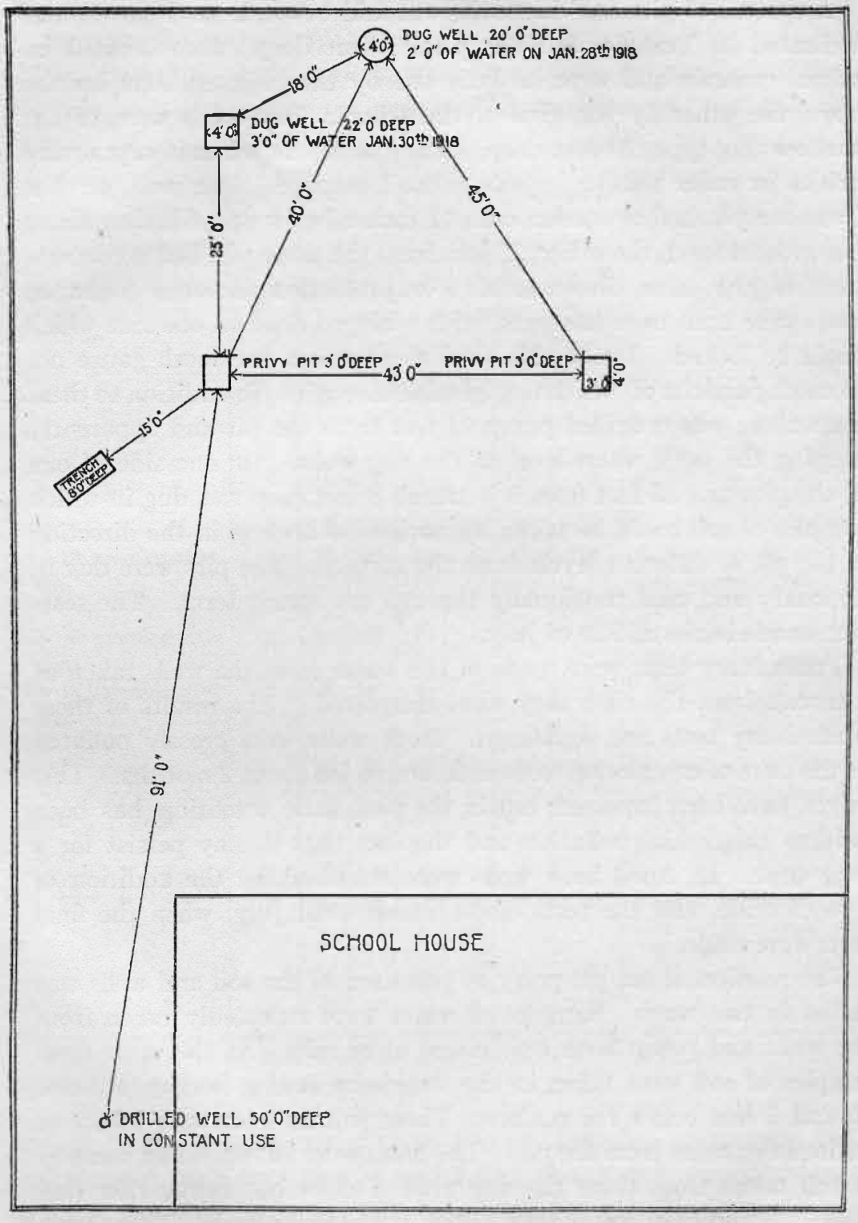
Character of soil.	Samples taken near pump.		Results: *B. coli* in.
	Dry side.	Wet side.	
Sand-clay.	1 ft. deep.		+ (1.0 gm.)
	3 " "		−
		2 ft. deep.	+ (0.1 gm.)
		6 " "	+ (1.0 gm.)
		9 " "	−

It is evident that in this instance the continued fall of water was capable of carrying the pollution through at least 6 feet of soil. In another similar instance, pollution was found at a depth of 4 feet. The soil was a compact sandy clay. In still another instance, in hard clay soil a sample of soaked soil near an open well, taken diagonally in the direction of the well and at a depth of 3 feet, was found to be contaminated in 0.1 gm. Of fourteen soil samples taken in the soaked ground near wells at a depth of 1 to 10 feet, eleven were found to be contaminated.

These observations indicate that the force of dropping water, especially if continued over a long period, may carry bacteria to a depth of at least 3 feet in hard clay and 6 feet in sand-clay soil. There is, therefore, a strong probability that continued heavy rains will accomplish the same results in a shorter time.

Experimental Pit.

In order to obtain, if possible, a conclusive answer to the question whether or not pollution from pits will penetrate to any appreciable distance through the subsoil, the following field experiment was devised.



TEXT-FIG. 3. Trenches and wells for soil pollution tests in a sand-clay soil at Penns Neck school, West Windsor Township, Mercer County, New Jersey.

A system of pits and dug wells was laid out in a sand-clay soil as indicated in Text-fig. 3. The pits, 3 feet deep, were located on school grounds and were in daily use by the children, one by the boys, the other by the girls of the school. The wells were of the shallow dug type, 20 feet deep. They were left without supporting bricks in order not to impede subsoil seepage. One well, 40 feet from one pit, had a wooden curb 12 inches below and 6 inches above the ground level, the other, 25 feet from the same pit, had a concrete curb of the same dimensions. For protection inverted V-shaped roofs were built over the wells, with a hinged door on one side which could be locked. Inside this shed there was a fine mesh gauze net to catch particles of dirt that might fall through. In addition to these wells there was a drilled pump 91 feet from the pit and apparently tapping the same water-level as the dug wells. On one side of one of the pits and 15 feet from it a trench 8 feet deep was dug in which samples of soil could be taken by horizontal borings in the direction of the pit at different levels from the surface. The pits were dug in February and used continually through the spring term. The tests were made in the middle of July.

Preliminary tests were made of the water from the wells taken at intervals from the time they were completed. The results of these preliminary tests are significant. Both wells were grossly polluted at the time of completion and continued so for about 2 months. This might have been expected, but in the past little attention has been paid to this initial pollution and the fact that it may persist for a long time. In April both wells were sterilized by the addition of hypochloride, and the tests discontinued until July, when the final tests were made.

The relation of the pit privy to pollution of the soil and wells was tested in two ways. Samples of water were repeatedly taken from the wells and pump both before and after rain. At the same time samples of soil were taken in the trench by boring horizontal holes $3\frac{1}{2}$ and 5 feet below the surface. These soil samples were taken at various distances from the pit. The holes were left open and samples of soil taken from them the day after a short but heavy rain had fallen. Samples of water were taken at the same time. These tests were repeated the next day. 3 days later soil samples were taken from

two new holes $3\frac{1}{2}$ and $4\frac{1}{2}$ feet, respectively, below the surface, as well as from the old shafts. Water samples were also taken.

The results of these various tests seem quite conclusive. *Before the rain* both dug wells contained *Bacillus aerogenes*, but not *Bacillus*

TABLE XXVI.

Pollution of Wells Near the Experimetal Pit.

Date.	Well No.	Amount of gas in lactose broth. 10 cc. of water.			Amount of gas in lactose broth. 1.0 cc. of water.		No. of bacteria per cc. at 37°C.	Remarks.
		1	2	3	1	2		
July 17; before rain.	1	28	40	25	0	15	20	*B. aerogenes* in 10 cc. samples, *B. sporogenes* in 1 cc.
" 17 " "	2	0	0	10	0	0	8	*B. aerogenes* in one 10 cc. sample.
" 17 " "	3	0	0	0	0	0	1	
" 18; morning after rain.	1	30	25	30	2	2	26	*B. aerogenes* in all 10 cc. samples; negative in 1 cc.
July 18; morning after rain.	2	45	20	30	0	0	13	*B. aerogenes* in all 10 cc. samples; negative
July 18; morning after rain.	3	0	0	0	0	0	1	in 1 cc.
July 19; after rain.	1	20	15	10	10	10	60	*B. aerogenes* in 10 cc. samples; negative in 1 cc.
" 19 " "	2	10	30	25	0	0	14	*B. aerogenes* in 10 cc.
" 19 " "	3	0	0	0	0	0	2	
" 23 " "	1	30	20	5	0	5	30	*B. aerogenes* in two 10 cc. samples; negative in 1 cc.
" 23 " "	2	0	8	5	0	5	12	No *coli*.
" 23 " "	3	0	0	0	0	0	1	

Well 1 was situated 25 ft. from the pit; Well 2, 40 ft. from the pit; and Well 3, pump on the ground, 90 ft. from the pit.

coli, in 10 cc., and neither of them in 1 cc. samples. Similarly, the soil samples from both the $3\frac{1}{2}$ and 5 foot shafts were free from *coli* in 5 gm. samples. *After the rain* both wells contained in 10 and 5 cc. samples *Bacillus aerogenes*, but no *Bacillus coli;* there was none in

TABLE XXVII.

Pollution of Soil at Various Distances from the Pit.

Date taken.	Sample No.	Depth from surface.	Distance from pit.	Gas in 5 gm. of soil; lactose broth.	Gas in 1 gm. of soil; lactose broth.	Gas in 0.1 gm. of soil; lactose broth.	Remarks.
		ft.	*ft.*	*per cent*	*per cent*	*per cent*	
July 17	1	3½	12	10	0	0	Soil: clay, little sand; aerobic spores; no *coli*.
" 17	2	3½	9	0	5	0	" " " " " " " "
" 17	3	3½	5	0	0	0	" " " " " " " "
" 17	4	5	9	0	2	0	Sand-clay soil, no *coli*.
" 18; after rain	6	3½	5	50	60	—	*B. coli* in 5 gm.; *B. aerogenes* in 1 gm.
" 19	7	3½	11	10	5	—	*B. aerogenes* in 5 and 1 gm.
" 19	8	3½	7	30	70	—	*B.* " " 5 " 1 "
" 19	9	3½	5	50	20		*B.* " and *B. coli* in 1 gm.
" 18	10	5	5	80	0		*B. sporogenes;* putrid odor; no *coli* or *aerogenes*.
" 19	11	3½	2; between pit and well.	30	70		*B.* " *coli* absent.
" 23	12	3¾	11	2	0		New shaft; no *coli*.
" 23	13	3¾	9	12	20		" " *B. sporogenes;* no *coli*.
" 23	14	4¼	12	0	70		" " *B.* " " "
" 23	15	4¼	9	40	0		" " *B.* " " "
" 23	16	5	9	20	5		" " *B.* " " "
" 23	17	3½	9	25	5		Old shaft; *B. coli* and *B. aerogenes* present.

1.0 cc. At the same time the soil samples taken from the shaft $3\frac{1}{2}$ feet from the surface were all contaminated with *Bacillus aerogenes* or *Bacillus coli* in $2\frac{1}{2}$ gm., whereas those taken from the 5 foot shaft were free from *Bacillus coli*, but contained *sporogenes*. 3 days later soil samples taken from new shafts were all free from *coli*, although the old $3\frac{1}{2}$ foot shaft still contained *Bacillus aerogenes* in 5 gm. samples.

The complete data are given in Tables XXVI and XXVII. There is relatively little doubt as to their interpretation. There is still the possibility that the increase in pollution after the rain may have been due to subsoil seepage. This explanation is, however, hardly in accord with the results as a whole. To begin with, the increase of pollution in the wells was comparatively small. At no time were true *Bacillus coli* found in the water even after the rain. Moreover, in the $3\frac{1}{2}$ foot shaft, even those samples taken 12 feet from the pit showed pollution, with the additional interesting fact that only one of the soil samples in this shaft contained true *Bacillus coli* and that was taken 5 feet from the pit. The soil from the 5 foot shaft, on the other hand, as well as that from new $3\frac{1}{2}$ and $4\frac{1}{2}$ foot shafts contained neither *Bacillus aerogenes* nor *Bacillus coli*. The shaft at $3\frac{1}{2}$ feet below the surface was thoroughly wet, while the others were only slightly damp.

It seems apparent then that what happens in dug wells is comparable to what occurred in the shaft nearer the surface. The water will have a tendency to move in the direction of least resistance, carrying with it polluting organisms from the surface. The direction is naturally that of gravity where the opening is parallel to the surface. When the shaft is perpendicular to the surface the direction of the water will be the diagonal or resultant of the two forces. For this reason even a concrete curb of only 1 foot is not sufficient to protect the well, since the heavy rain can apparently easily carry pollution for $3\frac{1}{2}$ feet when the pressure is released. This explanation accounts also for the results on well pollutions to be recorded below.

Relation of the Privy to the Purity of the Well Water.

In addition to the attempt to trace directly the passage of privy material in the soil, a study was made of the correlation between the type of privy and the purity of the well water. It was hoped that

such data would throw some light on the source of pollution. No previous report of a similar study has to my knowledge been made. Over one hundred open bucket and shallow drilled or driven pump wells were studied. These were located in different kinds of soils and near various types of privies. Like the soil tests, these comprise two series, one taken before and the other during the rainy period, but unlike those tests, there was little difference in the results.

TABLE XXVIII.

Correlation between the Purity of the Well Water and the Type of Privy.

Type of well.	No. of wells.	Water test for *B. coli*.				Type of privy.				
		− in 10 cc.	+ in 10 cc.	+ in 1 cc.	+ in 0.1 cc.	Pit.	Pail.	Septic tank.	Open.	None.
*Open bucket	28	1	4	16	7	11	4	2	9	2
*Shallow pump	32	17	3	7	5	22	1	1	8	0
Spring	1	1								
							Septic hole.			
Open bucket	35	0	5	14	17	34	1			
Pump	6	4	1	1	0	5	1			
Artesian	1	1								

Actual number of wells studied.

	Pumps.		Bucket wells.	
	No.	Per cent.	No.	Per cent.
Total	29	100	60	100
Not polluted	20	70	1	1.6
Polluted	9	30	59	98.4
" near bucket well	4	14		
" only 14 feet deep	3	10		

* In each type some of the wells were tested more than once; the results were as follows:

Repeated tests.

Pump.		Bucket well.	
Positive once, negative once	2	Positive twice	3
Negative twice	3		
Positive twice	2		
Positive three times	2		

Table XXVIII gives the results of the tests and the correlation between them and the types of privies. This table seems to show quite clearly that there is no relation between the type of privy and the purity of the water. On the other hand, there does seem to exist a definite relation between the type and condition of the well and the character of the water. The pumps, ranging in depth between 14 and 35 feet, were generally free from pollution, irrespective of the type of privy or distance from it; while the bucket wells, from 26 to 65 feet in depth, were invariably contaminated. The wells that were better protected (shed, curb, etc.) were less grossly polluted, the others were more so. There was no difference between wells near pits and those near pail closets or septic tanks. Among pumps that were found contaminated, four were only a few feet from an open well and of the same depth and consequently tapping the same water, three were only 14 feet deep, and one was near a septic pit, $7\frac{1}{2}$ feet deep, which was polluting the water-table 8 to 10 feet below the surface.

Non-Lactose-Fermenting Bacilli as Evidence of Source of Pollution.

The finding of *Bacillus coli* is generally accepted as a satisfactory index of pollution, but its presence gives no indication as to the source of the pollution. In fact we have no method at present that would enable us to differentiate human from animal pollution, though a suggestion of the possibility of developing such a method exists in the literature. The work of Smith and Moore, Savage, and others, for example, indicates that the non-lactose-fermenting gas-producing bacilli are relatively common in domestic animals—swine, calves, horses—and in mice, while they are relatively uncommon in human beings. It would appear, therefore, that a study of the relative frequency with which different types of non-lactose-fermenting bacilli appear in the stools of human beings and animals might lead to the development of a satisfactory index of the origin of pollution.

Although for the present the non-lactose-fermenting bacteria cannot be considered of any special significance, it was thought worth while to study the strains isolated from the polluted soils and wells in some detail. Organisms of this class were isolated from fifteen wells and fourteen polluted soils. These were studied culturally and serologically and the detailed results reported elsewhere.

The significant fact, as far as this investigation is concerned, is that the dominant type isolated from the polluted wells differed in its characters from that isolated from the soil. The strains isolated from the wells were mostly gas producers (13 out of 15); whereas most of those obtained from soils were non-gas producers (9 out of 15). That these results can be accepted as indicative of a difference in origin of the pollution cannot be claimed on the basis of the relatively few instances reported; but they are suggestive, and even significant when taken in conjunction with the results of the other experiments. A summary of the types isolated from each source is given in Table XXIX.

TABLE XXIX.

Non-Lactose-Fermenting Bacilli Isolated from Polluted Soils and Wells.

Source.	Dysentery-like.	Para-enteritidis.	Paracoli.	Morgan.
Wells	2	9	2	2
Soils	9	4	2	0

Summary of the Investigations on the Pit Privy.

The examination of over 50 pits in different soils during dry and rainy seasons brought to light a number of interesting facts.

1. In the cultivated area, surface soil, to a depth of 1 to 2 feet, is invariably polluted. Subsoil below this depth, except near a pit, is generally free from contamination. This makes it possible to trace pit pollution of subsoil by means of soil borings.

2. During dry seasons, whatever the age of the pit, pollution extends in a downward direction from the base of the pit for about 3 feet. This applies to clay, sand-clay, and sandy soils. The soil above the bottom of the pit, even 1 to 2 feet from the pit wall, is free from pollution, showing that there is no lateral extension of pollution.

3. During rainy seasons the pollution of the subsoil is more abundant, but the indications are that most of it is derived from the surface, the pit pollution extending to only about 5 feet from the pit. Sandy soils show more extensive subsoil pollution during rainy seasons than do other soils.

4. The study of the relation of the purity of the well to the type of privy substantiates the idea that the pollution from the privy does not penetrate through the soil. Most of the pollution in wells, in the soils mentioned, is apparently surface pollution. Driven or drilled pump wells were largely free from pollution. Open dug wells, on the other hand, were all more or less contaminated. The difference in the non-lactose-fermenting bacilli isolated from polluted wells and soils is a further indication that the well pollution is probably different in origin from that in the soil near pits.

5. A careful study of the soil and well pollution with experimental pits confirmed the deductions from the field studies. In this experiment there was no evidence of pit pollution in wells 25 and 40 feet from the pit. Soil tests showed that pollution may extend about 5 feet from the pit. The pit was used for 5 months by about 50 children.

6. These results and those obtained with two septic pits suggest that the important point to be considered in connection with the building of pit privies is the level of the ground-water. The vertical distance between the bottom of the pit and the ground-water is of greater significance than the horizontal distance between the pit and the well.

DISCUSSION.

These results and the inferences derived from them obtain further confirmation from two investigations which deserve to be reviewed in some detail. One was that conducted by Prof. Whipple in 1902, which came to my attention only a few months ago. Whipple studied the relation of pits to well pollution in the sandy soil of Long Island. He used the first of the two methods mentioned above; namely, he sank pumps at different distances from the latrine to various levels. His results are in striking agreement with those obtained in the present investigation. Some of his conclusions are quoted because of the clearness with which they are stated.

"These results indicate that . . . . the soil below a depth of 5 feet contained very small numbers of bacteria and was in fact almost sterile . . . . below a depth of 3 or 4 feet *B. coli* was invariably absent."

Speaking of subsoil pollution, he says:

"Water leaching through soil may carry dangerous germs for a distance that will depend in great measure upon the character of the soil and the velocity of flow. With

low velocity polluted water passing through for a distance of 25 feet may be considered as practically safe for use and it is probable that in most cases a distance less than this would serve as an efficient safeguard."

These conclusions refer, of course, to sandy soils. But the results of the present investigation show that they are equally applicable to clay or sand-clay soils, and since the velocity of flow in these soils is less than in sandy soils, the margin of safety is on the whole greater. This fact was clearly demonstrated by the laboratory experiments on soil penetration, as well as by the observations in the field.

Some of Whipple's experiments also tend to confirm the conclusion drawn from our own observations, that the distance of the base of the latrine from the water-table is of greater importance than the distance of the well from the privy. This is shown in Table XXX taken from Whipple's report. The water samples from the well 50 feet away were only slightly less polluted than those taken from the same depth 10 feet away from the latrine.

TABLE XXX.

Relation of the Pit Privy to Well Pollution in Sandy Soil (Whipple).

Latrine.	Distance of well from latrine.	Depth of well below water-table.	No. of samples of water tested.	Per cent positive in		
				0.1 cc.	1.0 cc.	10 cc.
Old.	10	2	12	8.3	16.7	41.7
	10	7	25	0.0	8.0	32.0
	10	17	16	0.0	6.3	18.8
	50	7	6	0.0	16.7	16.7
New.	10	2	16	0.0	6.3	12.5
	10	7	14	7.2	7.2	28.6
	10	17	15	0.0	6.7	6.7
	50	7	6	0.0	0.0	16.7

The other investigation which bears directly on our problem is the work of Eijken and Grijns on the Biological Activity of Tropical Soils, published in 1917. This report, which came to my attention after the work was completed, concerns the same general problem of soil pollution and in some respects follows the same general plan of attack. Pits were selected in different parts of the town and holes bored around these pits at various distances and to different depths to obtain either soil samples or samples of ground-water. The pits are very much like our own and varied in depth from 3 to 6 feet.

These authors, working in a tropical climate, completely confirm the results of this investigation. They found that in dry soil, *B. coli* was absent at ½ meter (20 inches) from the surface, but that during rain they can penetrate to a depth of 1½ meters (about 5 feet). They also conclude that pollution of the subsoil around pits is comparatively slight. In only one case were they able to trace the pollution to a distance of 5 meters (about 16 feet) from the pit.

"Where the ground water is not too high . . . . no contamination of the ground water occurs even very near the pits. . . . . Where the water-tight layer (of soil) is high and the ground water only a few decimeters from the surface, the surface infection is ordinarily the determining factor for the contamination of the ground water."

The close agreement of the results of these three independent investigations in different kinds of soil and under different climatic conditions warrants the conclusion that the pit contributes comparatively little to soil pollution; that such pollution rarely extends beyond a depth or distance of 5 feet from the pit, and that there is little danger of well pollution from pits provided the base of the latter is at least 10 to 15 feet above the water-table.

The Septic Type of Privy.

The septic type of privy differs in principle from the pit type in that the former is a wet, while the latter is a dry method of disposal. In all the various types proposed there is a receiving tank, partially filled with water, in which the excreta accumulate and undergo putrefactive decomposition. The overflow is collected in another tank and disposed of in various ways. The simplest form of this type of privy is the L. R. S. type, designed by the Public Health Service. The system consists of two barrels, and the effluent is sterilized either by chemicals or heat before it is disposed of. This involves a great deal of attention, unpleasant handling of the effluent, and the rapid deterioration of the barrels. A concrete system on the same design has been proposed as a substitute, but the handling of the effluent still remains.

A more elaborately designed system of this type, consisting of a septic, storage, and effluent chamber, respectively, with subsoil irrigation by means of unglazed tile pipes has been designed by the Kentucky State Board of Health. This privy has the advantages of the L. R. S. type; it also eliminates the necessity of handling of the effluent, but introduces the danger of possible pollution of the soil and ground-water.

The L. R. S. type was studied in the laboratory and the results on the viability of *Bacillus typhosus* and *Bacillus dysenteriæ* in the septic effluent and tanks are given above. The field studies were centered on the Kentucky sanitary privy. The investigation involved

an examination of (1) the character of the effluent; the color, odor, suspended solids, reaction, and presence of *Bacillus coli;* (2) the viability of pathogenic microbes in the effluent; (3) the nature and extent of the pollution of the surrounding soil; and (4) the pollution of the adjacent wells. Experiments were also made to determine the ability of typhoid or dysentery bacilli to pass from the septic chamber to the effluent pipe.

Methods.

Collection of Effluent.—The effluents were collected from the drain-pipe near the effluent chamber. This was easily accomplished by digging up the soil covering the tile, lifting a section of the tile, and allowing the effluent to run into a sterile bottle. When the tanks were in good working order a pail of water poured into the receiving chamber was sufficient to start the flow. In closets not properly cared for or leaking, 8 to 10 pails of water were sometimes required to start the flow through the pipe. The effluent thus collected was taken to the laboratory and subjected to the tests mentioned above.

Soil and Water Samples.—Soil and water samples were obtained in the manner described in connection with the pit privies. Soil samples were collected near and under the drain-pipes a short distance from the tanks.

Passage of Typhoid Bacilli.—The passage of typhoid and allied bacilli from the receiving tank to the effluent drain was tested by inoculating the former with a 24 hour broth culture of *Bacillus typhosus,* treating the tank as usual, that is, adding a pail of water at intervals, and examining the effluent for typhoid bacilli.

Viability Tests.—The viability tests were performed in the same manner as with the effluent from the L. R. S. privy.

Results.

Thirty privies of the Kentucky type were studied. Some of them had been in actual operation only 3 to 4 months, others 6 to 8 months, while a few had been installed 2 to 3 years prior to the tests. Most of the closets examined were of the rural type; five of them were in houses supplied with flush closets.

Character of Effluent, Color and Odor.—The effluent from the rural closets was light to deep brown in color and had only a mild fecal odor. When the septic tank was stirred up by the addition of a great deal of water, the odor of the effluent was strong. The effluent from tanks connected with flush closets was almost colorless and had only a slight odor.

Turbidity and Solids.—The turbidity and solids varied with the length of time the closets had been in operation. Some of those which had been in operation only 2 to 3 months contained solid particles of feces. Those closets which had been in use for 6 months or over gave a somewhat turbid effluent, but were free from large particles. The effluent from flush closets had no solid particles and was only slightly turbid.

Reaction and Colon Content.—The reaction determined by measuring the hydrogen ion concentration by the Sörensen colorimetric method varied from pH 7.4 to 8.0. The effluents from tanks that had been in operation 6 months or over had a pH value of 7.7–8.0 while those from tanks that had been in use for only 3 months varied from pH 7.4–8.0. The effluent from flush closets had a reaction of pH 7.2, due no doubt to the great dilution. All the effluents contained *Bacillus coli* in 0.01 cc.

Viability of Typhoid and Dysentery Bacilli in Kentucky Sanitary Privy Effluents.

Eight experiments were performed. Effluents having the same reaction were pooled and inoculated with broth cultures of typhoid and dysentery bacilli. The inoculated bottles were kept at room temperature ranging between 28.5° and 30°C. Tests were made immediately after inoculation and again at daily intervals. The results are summarized in Table XXXI.

The passage of pathogenic bacilli from the septic tank to the effluent pipe was tested in two ways. One closet was heavily seeded with a broth culture of *Bacillus typhosus*. Two pails of water were poured on top of that and the effluent was collected. The addition of water to the tank and collection of effluent samples were repeated at the end of 4, 6, 24, and 50 hours, respectively. In no case was the typhoid

bacillus recovered from the effluent. The experiment was repeated using fluorescine instead of a culture of *Bacillus typhosus* for inoculation into the tank. The dye appeared in the effluent after 24 hours. The dye evidently diffuses much more rapidly than do the bacteria.

TABLE XXXI.

Survival of Typhoid and Dysentery Bacilli in Kentucky Sanitary Privy Effluents.

Test No.	pH of effluent.	Survival of bacilli.	
		B. typhosus.	*B. dysenteriæ* Flexner.
		days	*days*
1	7.8	2	Not recovered on 2nd day.
2	7.5	2	1
3	8.1	1, not recovered on 2nd day.	1
4	8.0	1	0, not found after 24 hrs.
5	8.0	1	0, " " " 24 "
6	8.0	0, not recovered after 24 hrs.	0, " " " 24 "
7	7.8	4	1
8	8.0	3	1

Pollution of the Soil.—The results of the soil tests were identical with those obtained in connection with the pit privy. Eighteen soil samples were examined near six closets. Thirteen of these samples were taken directly under the drain-pipe and of these only four contained *Bacillus coli* in 10 gm. samples or less. Of the eight soil specimens taken 3 to 5 feet directly below the tiling, all but one were free from *coli*, though most of them had anaerobic spore bearers; whereas three of the five samples taken less than 3 feet below the tile contained *Bacillus coli*. Of those samples taken 2 feet from the tile and 2 feet from the surface, four out of five contained *Bacillus coli*. The detailed results are tabulated in Table XXXII.

The results of these soil tests were in such close agreement with those obtained in connection with the pit privy that it was not thought necessary to extend them. Like the soils tested before, these samples showed surface pollution to a depth of 2 feet below the surface. Similarly the pollution from the effluent pipe like that from the pits extended to a depth of about $2\frac{1}{2}$ feet and only rarely beyond that. The danger of subsoil pollution consequently appears to be rather small.

TABLE XXXII.

Extent of Soil Pollution from Kentucky Sanitary Privy Drains.

Sample No.	Distance from tile.	Depth below surface.	Depth below tile.	Results on	
				B. coli.	Anaerobic spore formers.
	ft.	*ft.*	*ft.*		
1	0	4	2½	−	+
2	0	6	4½	−	+
3	2	2	½	−	+
4	0	4	3	−	+
5	0	6	5	−	−
6	2	2	1	+	+
7	0	4	2½	+	+
8	0	6	4½	+	+
9	2	2	½	+	+
10	0	4	2½	−	+
11	0	5	3½	−	+
12	2	2	½	+	+
13	0	4	2	+	+
14	0	6	4	−	−
15	2	2	0	+	+
16	0	2½	1½	+	+
17	0	4½	3	−	+
18	0	6	5	−	+

Pollution of Shallow Wells.—The possibility of ground-water pollution was studied by examining the water of the wells on the premises. These tests indicate again that the extent of the pollution of the wells is not dependent on the character of the privy, but varies directly with the condition of the well itself. The better the well is protected against surface contamination, the purer the water; and the poorer the condition of the well, the less pure is the water. This relation is demonstrated by the details in Table XXXIII giving the nature of the well, its relation to the privy drain, and the colon content. It is also brought out strikingly by the summary table (Table XXXIV) showing the number of the different types of wells studied and their relative degree of purity. As a rule, the wells properly protected against surface contamination have a low colon content. Of eight cisterns tested the only one free from *Bacillus coli* in 10 cc. of the water had a tight cement cover; those with wooden covers all contained

TABLE XXXIII.

Relation of the Kentucky Sanitary Privy to Well Pollution.

Well No.	Type of well.	Depth.	Distance from drain.	Direction from drain.	Condition of well.	Results on *B. coli* in		
						10 cc.	1.0 cc.	0.1 cc
		ft.	*ft.*					
4	Cistern.		60	Same.	Brick-lined; concrete top.	−	−	−
8	"		40	"	Concrete; wooden top.	+	+	−
9	"		50	Down hill.	Brick-lined; wooden top.	+	+	+
21	"		50	Opposite.	Concrete; brick near pipe.	+	−	−
26	"		150	"	Brick-lined; wooden top.	+	−	−
28	"		75	"	Concrete; " "	+	+	+
29	"		50	"	" " " cracked.	+	+	+
30	"		60	"	" " "	+	+	−
2	Driven pump.	35	100	"	Good.	+	−	−
11	Drilled "	60	60	"	Curb near pipe loose.	+	+	+
18	" "	60	75	"	Stagnant water around pipe; leak to well.	+	+	−
25	Bored "	25	150	"	Concrete cover.	+	−	−
27	Dug "	25	150	"	Brick-lined; board top.	+	−	−
5	" well.	22	40	"	Poor.	+	+	+
6	" pump.	15	120	"	Open top.	+	+	+
7	" "	15	200	"	Cover in bad condition.	+	+	−
8a	" bucket.	12	125	"	Poor.	+	+	+
9a	" "	18	350	"	"	+	+	+
31	" pump.	12	50	"	Banked up; no top.	+	+	+
31 (2)	" "	15	100	"	In field; wooden top; open.	+	+	+
32	" wooden pipe.	8	100	"	Open top.	+	+	+
33	" bucket.	15	120	"	Brick-lined; open top.	+	+	+
3	" pump.	16	60	"	Fairly tight cover.	+	+	−

Bacillus coli in 1.0 or 0.1 cc. The drilled wells were relatively purer than the dug wells. In the two drilled wells showing *Bacillus coli* in 1.0 and 0.1 cc. samples of the water it was demonstrated, as will be shown in Table XXXIII, that the pollution was derived from the surface. The dug wells invariably showed gross contamination.

TABLE XXXIV.

Summary of Relation of Type of Well to Extent of Pollution.

Type of well.	No. tested.	*B. coli* in		
		10 cc.	1.0 cc.	0.1 cc.
Cistern	8	2	3	2
Driven well	1	1	0	0
Drilled "	3	1	1	1
Dug well	11	1	1	9

Pollution of Wells from Privy Drains.—In order to determine more directly the possibility of subsoil pollution of the ground-water by the effluent from these septic privies, the following experiments were performed. Three drilled wells, Nos. 11, 18, and 25, were selected for this purpose. Wells 11 and 18 each supplied water to about a dozen families and were located within 50 to 150 feet of as many septic privies. Both wells were more or less polluted. The conditions were therefore most favorable for the purpose of the experiments. Well 25 was on school ground within 150 feet from two Kentucky sanitary privies, one used by the boys, the other by the girls. The privies had been in use for about 2 years by over 100 pupils. The water of this well was also slightly contaminated despite the fact that the cover was solid cement sloping away from the pump. In the one case the large number of closets in use about 8 months, in close proximity to one another, caused massive contamination of the soil; in the other the long continued use (2 years) by a large number of children produced the same effect. If subsoil pollution of ground-water does occur, it seemed that sites selected should furnish the evidence of that fact.

In order to demonstrate the possibility of the passage of septic fluid from the privies to the wells the following procedure was adopted. A concentrated solution of fluorescine in 5 per cent ammonia water

was made and 100 cc. of this solution were added to a pail of water used for inoculation. One pail was added to each of the receiving tanks, and another to each of the tile drains. Five to ten pails of water were then added to the septic tank. This was repeated several times at intervals of 2 or more days. The use of the closets was continued as usual with the daily addition of a bucket of water to the tank. Samples of the water were taken at intervals, and the people were requested to report the appearance of a greenish tinge in the water.

The tests were started on June 3. Four closets nearest to Well 11 and four surrounding Well 18 were treated as outlined. On the school ground the two closets received double the amount of the dye. The water of the 3 wells was examined on the 4th, 6th, and 11th days after the addition of the fluorescine, always with negative results. At no time was the presence of the dye observed even after prolonged pumping.

The experiment was then modified in order to determine the possibility of surface contamination. 20 cc. of the concentrated dye were poured on the ground near the pumps and around the bolts fastening the pump casing. The pump was then worked intermittently for about half an hour. At the end of that time the dye appeared in pumps, Nos. 11 and 18, and continued to appear for several hours afterward. In Pump 25 (the school pump), the dye could not be detected in the well water. It was possible, therefore, to establish the likelihood of surface contamination in two of the three wells. Well 25 is especially interesting since despite the fact that neither surface nor subsoil pollution could be demonstrated, the water obtained after prolonged pumping was decidedly more turbid and more grossly polluted than at the beginning of the pumping. The explanation of this curious phenomenon probably lies in the fact that the Kentucky soil is of limestone formation and that once the limestone stratum is penetrated (as was the case in this well) pollution may be brought by a channel from some remote point. This would harmonize with the occasional appearance of a leaf or other bit of foliage in the water, especially after prolonged pumping.

Summary of the Studies on Kentucky Sanitary Privy.

1. The Kentucky sanitary privy if properly constructed and properly operated gives a darkly colored slightly turbid effluent free from solid fecal matter after it has been in use about 6 months. This effluent has a reaction of pH 7.7–8.0 and is rich in *Bacillus coli*. *Bacilli dysenteriæ* and *Bacillus typhosus* inoculated into the effluent die out within 1 to 4 days, respectively, if the mixture is kept in the dark at 28–30°C.

2. Experiments designed to demonstrate (*a*) the ability of typhoid bacilli to pass from the septic chamber to the drain-pipe in a few days, and (*b*) the subsequent passage of pollution from the drain to the well gave negative results.

3. Tests of soil specimens and of well waters confirmed the results obtained in connection with the studies of the pit privy. Pollution from the tiles does not, as a rule, penetrate the soil to a depth greater than 3 feet. The extent of the pollution of well water depends more on the condition of the well than on the type of privy or its proximity to the well.

These deductions may also reasonably be applied to other privies of the same class, provided they are so constructed as to comply with the conditions of these experiments.

Chemical Closets.

Within the past few years a type of privy known as the "Chemical Closet" has appeared on the market. These closets are supposed to accomplish two things: the disinfection and at least partial disintegration of the excreta. They usually consist of a can or tank which can be installed in the cellar or other part of the house, and is connected to a toilet in the bedroom. The receiving tank is charged with a concentrated solution or emulsion of the active chemical—usually caustic with or without a coal tar disinfectant—which breaks down the solid excrement and at the same time destroys the dangerous bacteria. When the tank is full, the contents are removed and buried or otherwise disposed of.

The device has a number of advantages. The tank is water- and fly-tight; the closet can be installed in the house, affording the desired

privacy and convenience. It requires no attention except when the tank is filled. The only question to be considered is whether disinfection is actually accomplished.

In the course of the investigation, opportunity was afforded to examine a number of closets of this type. All of them were made by one firm. The chemical used was caustic. The closets tested had been in operation for periods varying from 6 weeks to 3 months. The tank had a capacity of approximately 125 gallons and was supposed to require emptying about every 6 months.

TABLE XXXV.

Test of Fluid of Chemical Closet.

Source.	Time in operation.	Odor.	Color.	Solids.	Reaction (alkalinity).	Coli.	Anaerobes.
					N		
T	6 wks.	Pungent.	Deep brown.	Slight.	1.25	—	—
B	6 "	"	" "	"	1.5	—	—
S	6 "	"	Light "	"	1.75	—	—
St.	3 mos.	Very pungent.	Deep "	Small particles.	0.06	—	+ 0.1 cc.
C	2 "	" "	" "	Small particles.	1.5	—	—
G.C.	3 "	" "	" "	Small particles.	0.65	—	—
Gr.	3 "	" "	" "	Small particles.	0.5	—	—
St.	2 wks., recharged.	Pungent.	" "	Small particles.	1.0	—	—

The following tests were made on the material obtained from the tank: (1) odor; (2) presence of solid feces; (3) reaction; and (4) presence of *Bacillus coli*. Eight closets were examined with the results indicated in Table XXXV.

It appears from these results that this particular type of closet accomplishes what is claimed for it, during the first 3 months. In one of the closets in use 3 months, the alkali was exhausted to the point where it no longer killed the spore-bearing anaerobes though

it still destroyed the non-spore-forming bacteria. It would seem that the efficacy of the system could easily be controlled by a simple titration of the tank contents.

GENERAL SUMMARY OF RESULTS.

The investigation reported in the preceding pages brought to light the following facts, some observed for the first time, others confirmatory of previous observations.

1. The typhoid and dysentery bacilli succumb rapidly on exposure to an unnatural environment. (*a*) Both typhoid and dysentery bacilli die out in 1 to 5 days in septic tanks. (*b*) In solid feces the typhoid bacilli may survive for a period of 10 to 15 days, while the dysentery bacilli rarely survive longer than 5 days. The paratyphoid bacilli are the most resistant members of the group; the Shiga dysentery bacillus is the most sensitive. (*c*) The survival period of these organisms in soil is greater than in either feces or septic fluids, and varies particularly with the moisture and reaction of the soil. Temperature effects the viability, but the two main factors normally are moisture and reaction. In moist natural soil of a pH value of 6.6–7.4, the typhoid and dysentery bacilli may be recovered up to 70 days. In the same soil dry, the bacilli are not recovered after 2 weeks. In moist acid soils, pH 4.8–5.4, 90 per cent of the inoculated bacilli die out within the first 10 days, the others may survive as long as 30 days. All the organisms survive longer near freezing temperature (4°C.) than at higher ones (20–37°C.). (*d*) The antagonistic action of soil bacteria on typhoid and dysentery bacilli is due largely to the alkaline reaction resulting from their metabolism. Specific inhibitive substances are, however, elaborated by some soil bacteria, notably *Bacillus fluorescens* and *Bacillus proteus*.

2. The spread of pollution from a focal point is limited in scope. (*a*) Typhoid and dysentery bacilli under experimental conditions were not observed to spread laterally to any appreciable extent, although they were carried vertically through a column of 2 feet of porous soil. In denser soil they failed to penetrate through 1 foot. (*b*) In the field, where the subsoil was free from pollution, either near pit privies or near tile pipes from septic tanks, contamination extended downward to a depth of 5 to 3 feet, and laterally

only about 3 feet, from the bottom of the pit or tiles. (*c*) Heavy rains or constant dripping of water may carry surface pollution to a depth of 10 feet.

3. Pollution of wells is usually surface in origin. (*a*) There was no correlation between the type or proximity of the privy to the degree of contamination of the adjacent wells. The purity of the well water varied rather with the condition of the well. Driven shallow wells with pumps were, as a rule, free from contamination, while dug wells with pumps or buckets were generally grossly polluted. (*b*) Experiments with fluorescine failed to show subsoil pollution of wells from privies, but proved in some instances at least the possibility of surface contamination.

CONCLUSIONS.

It is evident from the results obtained in this investigation, as well as from those reported by Whipple and by Eijken and Grijns, that the pit and the septic privies, if properly constructed, are practically free from danger as far as the spread of intestinal infections of bacterial origin is concerned. Whether that holds true with regard to hookworm infection still remains to be determined. These privies might under certain conditions become a menace from a sanitary standpoint. If, for example, it was found that the soil is favorable to the survival and passage of the bacteria to the underground water, then they might be considered dangerous. However, under the various conditions represented in this investigation, there was neither prolonged viability nor ready passage through soil. In fact, the dangerous organisms were found to die out rapidly in feces and privy material, and water leaching through soil could, as a rule, carry them only a distance of about 3 to 5 feet. It seems, therefore, reasonable to conclude that these systems of sanitary disposal of human waste are practically safe, provided they meet the necessary requirements.

From the practical sanitary standpoint, there are certain requirements which the sanitary privy should fulfill. Since subsoil pollution is uncommon while surface pollution is widespread, the first obvious requisite is concentration of the excreta at a single point below the surface of the soil. The next essential is to prevent the mechanical

distribution of the fecal matter, either by overflow wash or by flies. The excreta should, therefore, be deposited a sufficient depth below the surface to prevent overflow during heavy rains and should furthermore be properly covered to eliminate fly breeding. Furthermore, since it has been shown that pollution may at times, under extreme conditions, penetrate a depth of 10 feet, it is important that a vertical distance of at least 10 to 15 feet be allowed between the fecal deposit and the ground-water level. Finally it is desirable that the privy require little attention and be relatively cheap.

The above generalizations should form the basis for the practical construction of either pits or septic privies. The pit privy can be used safely in any soil similar in character to those studied in the course of this investigation, provided the ground-water level does not rise higher than 10 to 15 feet from the surface. In such soils a pit about 3 feet deep would be within the limits of safety if properly protected against flies. The cheapness and the relatively little care that it requires would recommend it in certain communities.

In limestone regions and in soil where the water-table is near the surface, the pit privy should not be considered safe. In such localities the Kentucky sanitary privy or one of similar design might be recommended. This type of privy should have a sufficient storage capacity to allow time for the destruction of pathogenic bacilli—approximately 5 days. If that precaution is taken there should be very little danger of infectious material passing from the drains to the ground-water, especially if there is a layer of soil of about 5 feet between the drain and the ground-water level. The drain-pipe should also be placed about 2 feet below the surface.

In general, it should be emphasized that any form of subsoil disposal should be designed with a knowledge of the character of the soil and particularly with due regard to the ground-water level. The vertical distance between the source of pollution and the ground-water is the significant factor. The horizontal distance between the source of pollution and the well is of relatively slight importance except when there are underground channels or cracks in the soil.

This leads to a consideration of the character of the drinking water in the rural community. Although rural sanitarians have paid a great deal of attention to the proper disposal of human excreta, they

have practically overlooked the other important sanitar problem—the supplying of pure water. This investigation shows fairly conclusively that pollution of the water supply is mainly surface in origin. The dug bucket well is constantly exposed to the danger of surface pollution and direct human contamination. The question is of sufficient importance to warrant shifting some of the emphasis from the proper and safe disposal of the excreta to the protection of the water supply from direct human contamination. The danger from the latter source is real, while that from subsoil contamination is rather remote.

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