Public Health Reports

Vol. 65 • JULY 28, 1950 • *No. 30*

Effect of Formaldehyde on the Direct Microscopic Count of Raw Milk

By B. S. LEVINE, Ph. D.*

The addition of formaldehyde to milk samples intended for direct microscopic examination has never been included in Standard Methods as an explicitly recommended procedure. In the eighth edition of Standard Methods (*1*) the following statements appear: "Icing of samples may be omitted where chemical preservatives are used; for example, a drop of a 40 percent formaldehyde solution to each 10 ml. of milk in the sample bottle. Add the preservative either to the milk as the sample is taken or to the empty containers a few hours before use." In 1944 Robertson (*2*) made a preliminary study of formaldehyde-preserved milk samples, because, as he stated: "Some workers have questioned the desirability of adding formaldehyde to milk samples at the time of sampling . . ." and because of ". . . the alleged failure of the bacteria in the formaldehyde-treated milk to take the methylene blue stain so that they could be counted, using the direct microscopic methods."

The basic observations made by Robertson are worthy of being quoted at some length: ". . . the micro-appearance of the films suggests a formaldehyde-casein complex which is so porous that it permits the dye to penetrate irregularly and more deeply into the milk solids. The formaldehyde-casein complex apparently retains larger portions of the fat than is retained in films prepared from nonformaldehyde-preserved samples. Once the dye has penetrated the interstices, it is not so readily removed by washing or destaining, and consequently the intensity of the retained blue color increases the difficulty of identifying bacterial cells. This difficulty is so great as to make the satisfactory examination of smears made from formaldehyde-preserved samples, at least in some cases, a practical impossibility."

The observations of Robertson may have been largely responsible for the fact that reference to the use of formaldehyde as a preservative for milk samples to be studied by the direct microscopic procedure

*Bacteriologist, Public Health Service, Environmental Health Center, Cincinnati, Ohio. This report amplifies remarks presented before the Ohio Branch, Society of American Bacteriologists, Columbus, Ohio, Dec. 3, 1949.

has been deleted from the ninth edition of Standard Methods for the Examination of Dairy Products (*3*). Despite this deletion, however, occasional inquiries are received concerning the proper procedure to be followed in preserving milk samples by the addition of formaldehyde. Such inquiries indicate that in some State and municipal laboratories the preservation of milk samples seems necessary. For this reason, the author has studied the effect of formaldehyde on the direct microscopic count in greater detail than is reported by Robertson.

Procedure

Raw milk samples were collected early in the morning as deliveries were being made at the receiving platforms of milk plants. Collections were made in 50 ml. sterile sample bottles. They were brought promptly to the laboratory iced as prescribed in Standard Methods. As controls, two series of untreated milk films were prepared in triplicate on 1″ x 3″ glass slides to be stained as described later. Varying strengths of commercial or U.S.P. formalin (40 percent formaldehyde) were then added to sets of sterile vials as follows:

Set No. 1—0.1 ml. of ½ strength formalin, equivalent to 0.2 percent formaldehyde concentration after the addition of 10 ml. of the milk.

Set No. 2—0.1 ml. of ⅕ strength of the original formalin, constituting 0.08 percent formaldehyde concentration on the basis of 10 ml. of the milk.

Set No. 3—0.1 ml. of 1/10 strength of commercial formalin, constituting 0.04 percent formaldehyde concentration.

Set No. 4—0.1 ml. of 1/20 strength of formalin, constituting 0.02 percent of formaldehyde on the basis of 10 ml. of the milk sample.

Upon the addition of the 10 ml. of the corresponding milk samples to the vials containing 0.1 ml. of the desired strength of formalin, they were shaken 30 times to insure proper distribution of the formaldehyde in the milk. Two series of triplicate milk film slides were prepared from each of the four sets at time intervals indicated in the following paragraphs. One series of the milk film slides was stained with the optional Standard Methods stain, containing 30 ml. of 95 percent ethanol, denoted hereafter as the A-M-B stain (alcohol-containing methylene blue). The other series was stained with the acid- and water-free stain, denoted hereafter as the A-W-F stain.[1]

Effect of 0.2 Percent Formaldehyde Concentration

As stated, a 0.2 percent formaldehyde concentration is obtained when 0.1 ml. of ½ strength or 0.05 ml. full strength commercial formalin is added to 10 ml. of milk. Since a drop is roughly equivalent to a minim, or 1/20 ml., a 0.2 percent concentration of formaldehyde is practically the same as that obtained by adding 1 drop of

[1] The significance of the data as originally obtained was determined by statistical analysis made by John S. Wiley, Senior Sanitary Engineer of the Environmental Health Center, to whom the author expresses his grateful acknowledgment.

the full strength formaldehyde to 10 ml. of milk, as was mentioned in the eighth edition of Standard Methods. Two series of triplicate film slides were made of this set of milk samples prior to the addition of the formaldehyde and at the following time intervals after the addition of the formaldehyde: (1) within 2 hours, (2) after 3 days, and (3) after 8 days storing at room temperature. One series was stained by the optional Standard Methods formula; the other series was stained by the A-W-F staining procedure. A minimum of 30 microscopic fields in each of the triplicate films was counted for each milk sample studied. The final numerical estimates recorded in any of the following tables thus represent an average of not less than 90 fields counted over three separate milk films.

The effect of 0.2 percent formaldehyde concentration on the general microscopic appearance of the stained milk films has been aptly and correctly described by Robertson. A few points from additional observations should perhaps be recorded here. There appeared a general tendency for the milk proteins to stain more intensely, and for the organized cells, especially the bacteria, to stain considerably less intensely than in the untreated milk. In films stained by the optional Standard Methods stain, cracks extending in an astral formation around the fat-removed vacuoles were prominently in evidence and enhanced the difficulty in counting the milk films. These effects of 0.2 percent formaldehyde on the stained milk films became evident within 2 hours of the addition of the chemical. They became more pronounced as the storage time was prolonged. At the end of 8 days it became extremely difficult to make the counts, and about 35 to 40 percent of the stained films could not be counted. For films stained by the A-W-F procedure such effects were markedly reduced, but they were still of sufficient magnitude to prevent proper counting of stained cells. Averages of triplicate counts made on such a set of 30 milk samples are presented in table 1. Values derived for the mean, the standard error of mean, and the interpretation of the significance of difference between means are presented at the end of each column and between corresponding columns of table 1. The interpretation in terms of not significant, high, and very high are considered self-explanatory.

It seemed desirable to determine the effect which counts obtained on milk samples to which formaldehyde was added to a final concentration of 0.2 percent may have upon practical grade placement of milk. For this purpose the data presented in table 1 were analyzed on the basis of the number of milk samples yielding direct counts of 200,000 or less per ml., as is shown in table 2. In the untreated condition of the 30 samples, 8, or 26.7 percent, gave counts of 200,000 or less by the A-M-B stain. By the A-W-F stain only two samples, or 6.7 percent, gave counts of 200,000 or less. Within 2 hours after

Table 1. *Effect of 0.2 percent formaldehyde on the direct microscopic count of the raw milk: Average counts, in thousands, of three milk films per specimen (counting 30 microscopic fields per film)*

Specimen No.	Standard alcohol-containing methylene blue stain				Acid-and-water-free methylene blue stain			
	Untreated, stained immediately	Formaldehyde added, stained after			Untreated, stained immediately	Formaldehyde added, stained after		
		2 hours	3 days	8 days		2 hours	3 days	8 days
1	190	180	190	Unreadable	390	350	270	190
2	370	240	230	180	420	440	390	480
3	300	300	300	Unreadable	430	410	350	350
4	530	580	510	100	760	600	580	430
5	150	150	110	80	200	210	150	220
6	200	100	120	50	490	390	300	240
7	230	160	160	Unreadable	450	350	270	120
8	200	180	120	Unreadable	400	240	320	170
9	280	170	180	50	370	350	300	170
10	240	190	100	70	400	340	300	100
11	550	550	280	150	610	610	380	280
12	320	320	110	Unreadable	300	220	120	100
13	700	320	210	120	680	600	230	140
14	370	260	190	50	490	470	240	140
15	460	290	230	Unreadable	490	350	340	140
16	260	230	50	Unreadable	570	280	90	100
17	650	620	100	130	760	640	260	240
18	1,200	960	230	Unreadable	1,500	980	470	100
19	440	360	70	80	470	400	230	130
20	2,300	2,300	660	600	2,400	2,300	2,000	1,700
21	180	130	30	50	350	330	80	80
22	510	440	200	150	720	700	260	230
23	820	570	190	160	980	900	370	500
24	370	310	80	50	500	300	130	100
25	370	330	15	80	490	410	170	90
26	200	70	40	Unreadable	300	260	120	100
27	190	70	40	40	180	160	120	80
28	220	100	80	70	230	220	190	180
29	180	90	90	80	290	260	150	140
30	260	170	100	70	300	280	250	200
Mean	441.3	358.0	167.2	114.8	564.0	478.3	314.3	241.3
Standard error of mean	76.5	76.2	25.2	25.7	79.3	72.5	61.8	54.3
Significance of difference between means	not significant; very high; very high				not significant; high; very high			

the addition of the 0.2 percent formaldehyde, the number of milk samples yielding such counts by the A-M-B stain was 13, or 43.3 percent. The number in the A-W-F stained samples remained essentially unchanged. After 3 days' storage of the formaldehyde-preserved milk samples, the number yielding direct counts of 200,000 or less was 22, or 73.3 percent, by the A-M-B stain and 10, or 33.3 percent, by the A-W-F stain. As in our preceding investigations (*5*) the A-W-F stain proved superior to the A-M-B stain. It must be emphatically stated, however, that the counting of stained formaldehyde-treated milk films was considerably more difficult than counting similarly stained films in the control or untreated series.

The analysis presented in table 2 clearly shows that the addition of 0.2 percent formaldehyde may result in classifying milk of inferior quality as grade A. As previously stated, such concentration of

formaldehyde is equivalent to the concentration obtained when one drop of full strength formaldehyde is added to 10 ml. of milk. It would, therefore, not be out of place to express the following opinion at this point: The deletion from the ninth edition of Standard Methods of reference to the addition of one drop of full-strength formaldehyde to 10 ml. of milk appears to be well-considered and appropriate.

Table 2. *Effect on grading by direct microscopic counts of raw milk preserved by 0.2 percent formaldehyde*

	Total samples examined	Samples with counts of 200,000	
		Number	Percent
Standard alcohol-containing methylene blue stain:			
Untreated, stained immediately	30	8	26.7
Treated and stained in 2 hours	30	13	43.3
Treated and stained after 3 days	30	22	73.3
Acid- and water-free stain:			
Untreated, stained immediately	30	2	6.7
Treated and stained in 2 hours	30	2	6.7
Treated and stained after 3 days	30	10	33.3

Effect of 0.08 Percent Formaldehyde Concentration

A concentration of an 0.08 percent formaldehyde in milk was obtained by adding to a vial 0.1 ml. of ⅛ dilution of commercial or U.S.P. formalin (containing 40 percent formaldehyde) to which was then added 10 ml. of milk. Although a regrettably small number of milk samples was studied, the constancy of the results obtained, as recorded in table 3, appears highly encouraging. The unfavorable effects noted with 0.2 percent formaldehyde appeared in a far less pronounced manner. The differentiation between background and stained cells could be made reliably even after 8 days of storage at room temperature. It must be stated, however, that the counting of milk films so treated and stained required a greater degree of concentration and resulted in greater eye strain. Here again, the A-W-F stain was superior. It is possible, however, that some microscopists may prefer the darker appearance of the A-M-B stained films even though they tend to cause greater eye fatigue than the A-W-F stained milk films.

The values of the means and standard error of mean indicate that there is no statistically significant difference between the means in any two columns where either A-M-B or A-W-F stain is used. It is also apparent from this table that actual counts by the A-W-F stain were of greater magnitudes than the counts obtained by the A-M-B stain.

On the basis of the data presented in table 3, it is suggested (though not recommended) that if it is necessary to add formaldehyde to preserve milk samples for direct microscopic counts, it be done on the basis of adding 0.1 ml. of ⅛ dilution of U.S.P. formalin to 10 ml. of

the milk sample. It is hoped that other workers interested in the preservation of milk samples for study by the direct microscopic method will try to verify the results obtained here with an 0.08-percent concentration of formaldehyde in milk as reported in table 3.

Table 3. *Effect of 0.08 percent formaldehyde on the direct microscopic count of raw milk: Average counts, in thousands, of three milk films per specimen (counting 30 microscopic fields per film)*

Specimen No.	Standard alcohol, containing methylene blue stain					Acid- and water-free methylene blue stain				
	Untreated, stained immediately	Formaldehyde added, stained after				Untreated, stained immediately	Formaldehyde added, stained after			
		2 hours	1 day	3 days	8 days		2 hours	1 day	3 days	8 days
1	190	180	140	160	300	300	420	320	360	360
2	650	850	750	750	830	880	1,130	1,150	1,450	1,600
3	1,550	1,550	1,400	1,500	1,600	2,300	2,000	1,900	1,900	2,000
4	650	760	830	840	720	800	710	730	740	660
5	320	300	300	300	400	360	400	430	300	470
6	320	270	220	330	300	430	460	470	480	470
7	400	550	700	510	360	720	650	630	320	420
8	400	270	350	300	300	360	360	430	320	400
9	600	430	400	300	270	530	520	510	530	300
10	360	320	310	310	310	450	400	400	400	450
Mean	544.0	548.0	540.0	530.0	539.0	713.0	705.0	697.0	680.0	713.0
Standard error of mean	121.6	131.6	121.2	127.8	133.0	187.3	161.3	153.4	174.2	186.0

NOTE: There is no statistically significant difference between the means in any two columns where either A-M-B or A-W-F stain is used.

Effect of 0.04 and 0.02 Percent Formaldehyde Concentration

Reasoning from the previously reported results that a further reduction in the formaldehyde concentration might disclose a point at which suitable preservation conditions for milk samples could be arrived at, met with obstacles which might have been anticipated. It has been known for some time that bacterial species exist which not only grow but thrive in surprisingly high concentrations of formaldehyde. Recently Thompson and Dodd (*6*) worked with strains of *C. diphtheriae* which could not survive an initial 0.06 percent formaldehyde concentration. By a procedure of successive transfers to increasing concentrations of formaldehyde, they were able to develop a high degree of tolerance, so that the strains were able to grow well in as high a formaldehyde concentration as 0.1 percent. None of the resistant strains, however, could be cultivated in the presence of 0.2 percent formaldehyde.

With specific reference to the study under discussion, the following interesting and important observations were made. Two sets consisting of 25 milk samples each were studied. To one set 0.1 ml. of $\frac{1}{10}$ dilution and to the other set 0.1 ml. of $\frac{1}{20}$ dilution of formalin were added for every 10 ml. of the milk samples, making final formaldehyde concentrations of 0.04 percent and 0.02 percent, correspond-

ingly. A survey of the microscopic counts made on milk films prepared after 2 days of storing the treated samples at room temperature disclosed that in the majority of the milk samples there occurred a sudden falling off in the counts with both the A-M-B and A-W-F stains. In a few milk samples, however, the number of individual bacteria per micro-field was in excess of 200—more than there were originally. It was also observed that the originally seen types of heavily stained bacteria practically disappeared and that the now predominating lightly stained bacteria represented new and progressive growth occurring during the time of storage.

A study of milk films stained 4 days after storage at room temperature confirmed the previously recorded observations. In this instance, the number of specimens with progressively increasing counts was as high as 25 percent. None of the milk samples, however, showed any casein curds nor any marked thickening of the milk sample. Eight days following the addition of 0.04 and 0.02 percent of formaldehyde, 35 percent of the milk samples curdled, and the number of lightly stained, mostly spore-forming bacteria was beyond any possible counting. In those samples where curdling or thickening of the milk did not take place, the number of heavily stained bacteria was reduced to only a few, totaling not more than about 30 per ml. of the treated milk.

Further observations and preliminary studies for the purpose of obtaining a clue to the mechanisms of action leading to the above-reported phenomena indicated that the explanation may be a rather simple one. The progressive growth appeared to be due to the presence in the original milk samples of formaldehyde resistant species of bacteria, presumably of the thermophilic and thermoduric types of spore-forming and nonspore-forming variety. Preliminary experiments with differential stains suggested the possibility of the following effects of formaldehyde on other bacteria normally occurring in milk. The formaldehyde concentration apparently was strong enough to alter the permeability of bacterial surface and thereby impeded the processes of metabolism. However, the concentration apparently was not strong enough to penetrate into the cytoplasm of the bacteria. Reproductiveness was arrested, but the endoenzymes and other endogenous digestive agents remained unimpaired. Autolysis set in, and the bacteria disintegrated at a rapid rate. As applied to the direct microscopic counts, it can be stated that the two concentrations of formaldehyde studied were definitely too low for practical purposes of preservation.

Summary

The effect of several concentrations of formaldehyde on the direct microscopic counts of raw milk were studied. It was noted that in

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concentration of 0.2 percent or more the milk proteins of the background became brittle, showed an unevenness of spread, and acquired a higher affinity for the methylene blue dye. All cells, especially bacteria, lost in their power to adsorb the dye. This effect became evident within 2 hours of the addition of the formaldehyde. At the end of 3 and especially 8 days of storage at room temperature, these unfavorable effects became predominant. The direct counts either could not be made or their values were seriously impaired.

With concentrations of 0.08 percent formaldehyde, it appeared possible to make dependable direct counts after as long a period as 8 days storage at room temperature. However, the microscopic study of such milk films required a greater concentration on the part of the microscopist and consequently caused more rapid eye fatigue.

In concentration of 0.04 percent, formaldehyde-resistant bacteria rapidly proliferated, while bacteria normally occurring in raw milk rapidly disappeared.

Conclusions

1. The addition of one drop of full strength formalin for every 10 ml. of raw milk rapidly results in lowered direct microscopic counts. This procedure should not be resorted to even in instances of great emergency.

2. Where it is necessary to preserve milk samples, and in the absence of more appropriate methods of preservation, the results here reported indicate that 0.1 ml. of ⅓ dilution of formalin (40 percent formaldehyde) be added for every 10 ml. of milk, making a final 0.08 percent formaldehyde concentration.

3. Concentrations of formaldehyde appreciably below 0.08 percent should not be used to preserve raw milk for direct microscopic counts.

4. Formaldehyde is not the ideal preservative for milk samples intended for direct microscopic examination.

REFERENCES

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Field Tests of Molluscacides Against *Australorbis glabratus* in Endemic Areas of Schistosomiasis in Puerto Rico

By E. G. Berry,* M. O. Nolan,* and J. Oliver González †

Schistosomiasis has been recognized as a disease of world-wide importance even though it is not universal in its distribution. Certainly, with the philosophy of the economic interdependence of nations in mind it must be realized that a disease which hinders the economic development of such a country as Egypt, where schistosome infection is the most serious public health problem (*1*), adversely affects countries geographically remote from its focus. Moreover, with the changing conditions of travel, it becomes increasingly possible that new foci of the disease may be established.

Despite the fact that copper sulfate has been reported to be an effective molluscacide, the use of this compound over a period of many years has not resulted in adequate control of schistosomiasis. Reasons for this may be failure to apply adequate concentrations of chemical, and the ability of organic materials and plant life in treated waters to bind the available copper. The need for molluscacides of greater efficacy has been, therefore, increasingly recognized.

Within recent years, efforts have been made by several investigators to develop molluscacides for the destruction of the snail intermediate hosts of the human schistosomes. Reports of such investigations include those of Halawani (*2, 3*), Stirewalt and Kuntz (*4, 5*), McMullen and Graham (*6*), McMullen et al. (*7*), Pesigan and Masiluñgan (*8*), Jachowski and Stirewalt (*9*), and McMullen et al. (*10*). The most extensive field tests have been conducted by McMullen et al. (*10*) in Japan and have resulted in the finding that sodium pentachlorophenate and dinitro-*o*-cyclohexylphenol or its dicyclohexylamine salt were the most effective of all chemicals tested for the control of *Oncomelania nosophora*, the intermediate host of *Schistosoma japonicum*.

In the Laboratory of Tropical Diseases, Nolan and Mann (report to be published) have been engaged for several years in screening chemicals in an effort to develop more effective molluscacides. *Australorbis glabratus*, the intermediate host of *Schistosoma mansoni* in parts of the Western Hemisphere, has been employed in these tests. On the basis of some of these tests, certain promising compounds were tried

*Laboratory of Tropical Diseases, Microbiological Institute, National Institutes of Health, Bethesda, Md. †The School of Tropical Medicine, San Juan, P. R.

in field tests in the vicinity of Brownsville, Tex., in January 1949 by Nolan and Berry (*11*). These field studies were made on *Tropicorbis obstructus donbilli*, which is not an intermediate host of *Schistosoma mansoni* but which is closely related to *Australorbis glabratus*. Of necessity these field tests were made only in standing waters. Of the dozen compounds tested, pentabromophenol and sodium pentachlorophenate showed promise of being good molluscacides.

Further screening tests of other chemicals have been made in the Laboratory of Tropical Diseases since these first field tests were performed. Several additional compounds were found to kill *A. glabratus* within 24 hours in dilutions of 10 ppm or less. It was decided that these should be tested in the field and additional studies made on pentabromophenol and sodium pentachlorophenate in an endemic area of schistosomiasis and under a variety of aquatic conditions. Consequently, arrangements were made for conducting the tests in Puerto Rico.

Australorbis glabratus Say is widely distributed throughout Puerto Rico and is found, generally in large numbers, in many different types of aquatic environment such as streams, irrigation ditches, swamps, and lakes, whether relatively clear or grossly polluted. It is the largest planorbid species found in the island and is the only species known to serve as the intermediate host of *Schistosoma mansoni* in Puerto Rico. In areas where human pollution is present and this species of snail is found, the possibility of schistosomiasis is always a potential problem.

Materials and Methods

The compounds selected for field studies were chosen from among more than 750 chemicals which have been screened in the laboratory tests. The techniques of the screening procedures and the data regarding these studies are now in manuscript (Nolan and Mann). No attempt was made in the laboratory to simulate conditions that might prevail in natural waters; the compounds were tested in aqueous solutions uninfluenced by such variable factors as soil or mud, aquatic vegetation, or organic content. Dechlorinated tap water was used. It was borne in mind that the final evaluation of the compounds as molluscacides would have to be made in the field under the diverse conditions that would be encountered in widely separated areas. In the laboratory, compounds that were 100 percent effective against *A. glabratus* within a 24-hour period were arbitrarily considered to be of high potency and promising for field trial. Table 1 gives the name, structural formula, and source of the compounds which were employed in the field tests. Table 2 presents results obtained with these same chemicals in the laboratory screening tests.

Mammalian toxicity tests had been made on only three of these compounds. Therefore, to avoid risk to man and domestic animals,

Table 1. *Name, structural formula, and source of chemical compounds employed in field tests in Puerto Rico*

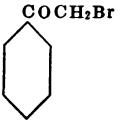
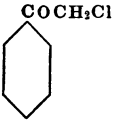
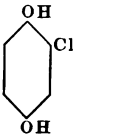
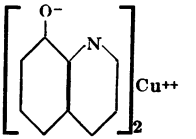
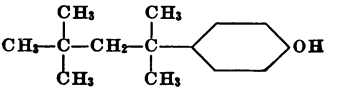
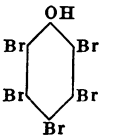
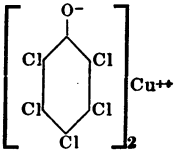
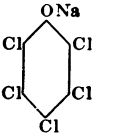
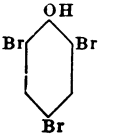
Survey No.	*Compound and supplier*	
490	Acetophenone, bromo- (Phenacyl bromide) Eastman Kodak Co.	
547	Acetophenone, chloro- (Phenacyl chloride) Eastman Kodak Co.	
501	1,4-Hydroquinone, chloro- Eastman Kodak Co.	
679	8-Quinolinol, copper salt Monsanto Chemical Co.	
585	Phenol, octyl- (Diisobutyl phenol) Rohm & Haas.	
246	Phenol, pentabromo- Dow Chemical Co.	
674	Phenol, pentachloro-, copper salt Monsanto Chemical Co.	
270	Phenol, pentachloro-, sodium salt Dow Chemical Co. ("Dowicide G") and Monsanto Chemical Co. ("Santobrite briquettes").	
555	Phenol, 2,4,6-tribromo- Eastman Kodak Co.	

Table 1. *Name, structural formula, and source of chemical compounds employed in field tests in Puerto Rico*—Continued

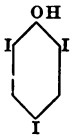
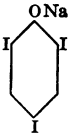
Survey No.	*Compound and supplier*	
566	Phenol, 2,4,6-triiodo- Eastman Kodak Co.	
626	Phenol, 2,4,6-triiodo-, sodium salt Eimer & Amend.	

Table 2. *Results of laboratory screening tests of chemical compounds employed in field tests in Puerto Rico*

Compound	Mortality of *Australorbis glabratus* (percent)					
	10 ppm	5 ppm	4 ppm	3 ppm	2 ppm	1 ppm
Acetophenone, bromo-	100	100	100	100	100	70
Acetophenone, chloro-	100	100	100	100	50	0
Hydroquinone, chloro-	100	100	90	80	60	
8-Quinolinol, copper salt	100					
Phenol, octyl-	100	60				
Phenol, pentabromo-	100	100	100	50	30	0
Phenol, pentachloro-, copper salt	100	100	100	100	100	90
Phenol, pentachloro-, sodium salt	100	100	100	100	80	10
Phenol, 2,4,6-tribromo-	100	100	100	100	50	0
Phenol, 2,4,6-triiodo-	100	100	100	100	100	10
Phenol, 2,4,6-triiodo-, sodium salt	100	100	90	40	10	0

it was necessary to select areas for testing which were relatively isolated. In order to make a comparative study of the 11 compounds it was also necessary to find a large-sized area heavily populated with *Australorbis glabratus* so that some of the chemicals could be tested in closely situated plots with adequate controls. Only one such area was found. This was a large temporary swamp, heavily populated with the snails, located just east of Vega Baja on Highway 2R. It was in a low area surrounded on three sides by sugar cane fields and devoid of habitation or domestic animals. The water had accumulated to a depth of 1 foot or more in certain sections through the excessive and continued precipitation during the winter months. Permission to treat this swamp was granted by the Insular Land Office which generously cooperated even further by fencing the area with barbed wire as an extra precaution to prevent stray cattle from entering. Additional tests with the compounds were done in other parts of the island, e. g., in backwash waters along the Rio de la Arecibo just east of Arecibo; in lily ponds between Barceloneta and Arecibo; in roadside ditches between Vega Baja and Durado and in a few other areas which will be described in connection with the discussion of certain chemicals.

Readings of pH and temperature were taken separately for each test. In the case of the swamp near Vega Baja, large sections between the treated plots were used as controls. In all the initial experiments in this swamp the water was treated with the chemical in solution or in a fine suspension in acetone or alcohol in dosages calculated to produce a concentration of 10 parts per million. "Sure-shot" spray guns powered by compressed air were used for the application.

Estimates of the effectiveness of the compounds were made by ascertaining the proportion of viable snails present in the area before and after the application of the chemical. In the large swamp, observations were made prior to and 24, 48, 60, and 72 hours after the spraying. Unfortunately the water in the large swamp evaporated within 2 weeks after the tests were made, preventing further observation for a comparison of residual effects. However, in other areas, described in discussions of particular molluscacides, the observation periods were of longer duration. In some of the very small areas it was possible to obtain an approximate count of the entire population of snails found in the water and on the banks and vegetation. Most of the chemicals were so rapidly lethal that the snails had no opportunity to bore into the mud. In the larger areas it was likewise possible to examine large numbers of snails for viability although of course the total population could not be counted. The effects of treatment of the water were generally so dramatic that no equivocation in the expression of the results is necessary.

Results

Representative data on the comparative value of the chemical compounds tested in the Vega Baja swamp and in other areas are presented in table 3. In repeat tests with some of the compounds,

Table 3. *Comparative data of 11 chemical compounds used as molluscacides in still water*

Compound	Solvent	Size (square feet)	Area treated		Temperature of water (C°)	Effect on *Australorbis glabratus* Mortality (percent)
			Mean depth (feet)	pH of water		
Acetophenone, bromo- (Phenacyl bromide).	Acetone	625	0.74	6.9	26	59
Acetophenone, chloro- (Phenacyl chloride).	Acetone	500	.84	6.7	25	20
Hydroquinone, chloro-	Acetone	474	.47	7.3	25	No effect
8-Quinolinol, copper salt	Alcohol	80	.55	8.0	32	25
Phenol, octyl-	Acetone	83	.38	7.1	32	10
Phenol, pentabromo-	Acetone	54	.35	7.2	25	95–100
Phenol, pentachloro-, copper	Alcohol + Tween 80	500	.93	6.9	28	95–100
Phenol, pentachloro-, sodium salt	Acetone	110	.24	7.6	29	95–100
Phenol, 2, 4, 6-tribromo-	Acetone	48	.32	8.0	32	95–100
Phenol, 2, 4, 6-triiodo-	Acetone + Tween 80	462	.41	7.6	30	95–100
Phenol, 2, 4, 6-triiodo-, sodium salt	Water	60	.38	7.4	28	95–100

results similar to those given in the table were obtained. These are not tabulated but will be discussed separately where appropriate.

It will be noted that 6 of the 11 compounds were highly effective against *A. glabratus*, producing a mortality of 90 percent or more. Certain compounds which killed *A. glabratus* in the laboratory when used in concentrations of 10 ppm or less (see table 2) were found to be noneffective or only partially effective in killing the total population of this species in the field. Chlorohydroquinone, octylphenol, 8-quinolinol copper salt, phenacyl bromide, and phenacyl chloride belong in this category. Phenacyl bromide showed a moderate effectiveness, with 59 percent kill of the snails, but this chemical as well as phenacyl chloride is a tear gas and very disagreeable to use, making it impracticable as a molluscacide.

The six effective chemicals, pentabromophenol, copper pentachlorophenate, sodium pentachlorophenate, 2,4,6-tribromophenol, 2,4,6-triiodophenol and its sodium salt, were successful in killing 90 to 100 percent of the snails when used in concentrations of 10 parts per million. Some of these have advantages over others and the various plots and conditions under which they were tested are discussed later. All of the tests, except where indicated otherwise, were conducted with concentrations of 10 ppm.

2,4,6-Triiodophenol was tested in two plots, one at the Vega Baja swamp and the other at a small pond at Arecibo. The phenol was dissolved in acetone and Tween 80 before dispersion. In both instances the compound was found to be an excellent molluscacide, producing 95 to 100 percent kill of the snails. The snails were found contracted but not dead at 24 hours, and dead at 72 hours. The compound was tested only in standing water, and since it apparently acts somewhat slowly, it might be less effective in flowing waters. It was the only effective molluscacide which seemed to have little if any effect on fish; no dead fish were found in either of the experimental plots following treatment. The sodium salt of triiodophenol was similar in its molluscacidal effects to the phenol. However, it was toxic to fish, possibly because of its greater solubility. The cost of these iodine compounds remains relatively high, which precludes their use in the near future as practical molluscacides.

2,4,6-Tribromophenol was tested in five different plots containing standing water. One test was run with the compound in a concentration of 20 ppm, the others at 10 ppm. Two of these areas were situated at Vega Baja in the area of stagnant drainage water described previously. The Hoffman Memorial pool at the University of Puerto Rico served for one test, and a pond on the road between Arecibo and Barceloneta for another. Two tests were performed in drainage ditches containing stagnant water on the road between Durado and Vega Baja.

The tribromophenol is readily soluble in acetone and was easily dispersed in that solvent by the spray gun. In all but one of the tests, application of this compound resulted in 95 to 100 percent kill. In two of the tests, not only was there a total kill of all the snails present, but the embryos within the egg membranes were dead within 48 hours. In one of the roadside ditches between Durado and Vega Baja, no live snails were found for 6 weeks after application of the chemical. The one exception, in which no kill was obtained, was the other roadside plot in this area. It is not possible for us to explain this aberration on the basis of any data collected; the area tested was merely another segment of the same ditch in which the disappearance of snails for 6 weeks was noted. Incidentally, it should be mentioned that it is believed the absence of snails from the latter area was due to a residual effect of the tribromophenol, since snails were abundant only 3 feet away from the treated plot, and there were no barriers to their movement into it. The compound was slightly more toxic to fish than the iodophenol. It had no visible effect on native vegetation although it did damage imported water lilies in the Hoffman pool. This latter area remained free of snails for at least 41 days after treatment, even though fresh water was allowed to run into the pool 3 days after treatment and continued to run during the period of observation. It may be presumed, therefore, that all snail eggs were killed by the chemical.

As the present market price of bromophenols is high, the use of this chemical as a molluscacide is not yet practical. However, it is possible that given a great enough demand, price reductions would ensue. It will first be necessary to demonstrate the efficacy of the compound in running water. All the tests reported here were done in standing waters.

Pentabromophenol was tested in three areas. One was a body of stagnant water at Arecibo, another a cement pool filled from a roadside drainage ditch between Durado and Vega Baja, the third a small pond off the road from Arecibo to Barceloneta. The kill was consistently 95 to 100 percent. Many snails migrated out of the water in the Arecibo test soon after the spraying and died within 48 hours. Pentabromophenol is lethal to fish but seemed to have little if any effect on aquatic vegetation. It is slowly soluble in acetone and alcohol, the two solvents available for these tests; hence more difficulty was experienced in dispersing it and a coarse jet was necessary on the sprayer. In addition to this disadvantage, this chemical has the same present obstacle of high cost as the preceding ones.

Copper pentachlorophenate was given three tests, one in the swampy area at Vega Baja and two in small lily ponds off the Arecibo-Barceloneta Road. This compound is very slightly soluble in 95 percent ethyl alcohol. It was used either as a fine suspension in alcohol or

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mixed with talc and a wetting agent, Alconox, and was found to be a very effective molluscacide, with kills of 90 to 100 percent within 24 hours of application. It has an advantage over other compounds in that it destroyed thick mats of algae and thus facilitated its own penetration into protected pockets of the ponds. It is, incidentally, also very toxic to fish.

Copper pentachlorophenate is low in cost and offers promise for development as an excellent molluscacide. Its greatest disadvantage is its insolubility in water. This difficulty may be overcome by mixing it with a water-soluble gum or wax. It is possible, also, that its insolubility may be turned to advantage in producing a long-lasting residual effect.

Sodium pentachlorophenate had been found to be a very effective molluscacide in the preliminary tests in Texas. It is also a relatively cheap compound and has the further advantage of solubility in water. Because of these considerations it was considered to be of particular promise. Two tests were made with it in standing water or in slow-flowing seepage water and some additional trials were made in various streams.

The test made in standing water was conducted in a small pond at Vega Baja. At 24 hours after treatment some snails were found alive, but all were dead at 48 hours. The compound thus was effective but it did not act as rapidly as the bromophenols or the copper salt of pentachlorophenol. Certain disadvantages were evident in its use: the compound is irritating to the mucous membranes and skin of the person applying it, especially when it is in powder form. However, it is also manufactured in pellets or 1-ounce briquettes, which minimize such effects. It was found very toxic to fish and killed guppies, *Lebistes reticulatus*, even at a concentration of 1 part per million.

Toxicity tests on rodents (*12*) had indicated that sodium pentachlorophenate was not too dangerous to use. However, before tests were made in flowing water some further assurance was necessary that the compound could be applied without injury to humans or cattle using the water below the site of application. Two toxicity tests were therefore conducted. In one, 200 cc. of water containing 20 ppm of sodium pentachlorophenate were administered by stomach tube to a 5-pound Rhesus monkey. The animal suffered no visible distress. In another experiment a calf was given water containing 20 ppm of the compound over a period of 4 days, during which time it consumed 40 gallons. No ill effects were observed. It was therefore concluded that little risk would be involved in applying sodium pentachlorophenate to water at concentrations of 10 ppm.

The first test was made in a seepage area at Patillas. Here the water was flowing very slowly, at about 9½ gallons per minute. Numerous pools choked with water hyacinths existed along the

course. Four ounces of sodium pentachlorophenate killed all the *A. glabratus* in the critical area, 20 feet long by 5.7 feet in width and 0.7 feet average depth; more than 90 percent of the snails for a distance of 120 feet below the application site were destroyed.

In the second plot, a ditch at Vega Baja flowing at the rate of 21.6 gallons per minute, 6.9 ounces of sodium pentachlorophenate were placed in a muslin bag and suspended in the water. All of the snails were killed for a distance of 320 feet below the application site. This effect was obtained in 24 hours.

The third test was performed at Los Peña, just east of Rio Piedras. Here a small creek (Quebrada Sabana Llana) combined most of the features desired for a critical test of the molluscacides in flowing water. In its course through the region chosen for testing, which was 660 feet in length, the creek cascaded over rocks in several narrow channels and in other places meandered slowly through pools 5 feet or more in depth. The average width was 1.8 feet and the average depth 0.4 feet. Current flow was 630 gallons per minute. Large populations of snails, principally *A. glabratus*, were found in the dense vegetation along the margins. It was expected that such a thick growth would probably deflect the flow of water containing the molluscacide and thereby furnish a rigid test of the efficacy of the compound.

The chemical was applied at a calculated dosage of 9.5 ppm based on a 6-hour flow rate. Four of the largest pools in the critical area were dosed separately at the rate of 10 ppm and the rest of the compound was applied by immersing it in four muslin bags in the stream at the head of the observation area. Two of the bags were placed in the rapid current and the other two along the margins where the current was reduced. Within 2 hours all the chemical had gone into solution in the swift flowing water and about half the contents had dissolved in the bags at the margins.

Two hours after application of the chemical, it was observed that many of the snails had contracted into their shells and hemorrhaging was seen in some of these. Twenty hours after treatment hundreds of dead snails were floating on the surface, and a very thorough search failed to reveal any live specimens in the critical area. Observations were therefore extended down the stream, and it was impossible to find any live snails for a distance of 1½ miles from the application site. Within this distance three tributary streams entered the creek. The chemical was not effective beyond the entrance of the third and largest tributary, probably because of the marked dilution beyond this point. Two days following treatment numerous egg clutches of planorbid snails were observed floating downstream. A number of these were collected and found to contain only dead embryos. Catfish (probably *Ameiurus nebulosus*), guppies (*Lebistes reticulatus*), and

eels (*Anguilla rostrata*) were dead within a few hours after application of the compound, although edible crayfish appeared unharmed. Algae in the stream turned yellow but other vegetation was not affected.

Discussion

The results of these tests indicate that certain halogenated phenols possess marked molluscacidal properties and are capable of destroying the snail population in infested waters when employed in relatively low concentrations. The pentachlorophenol salts give particular promise of practical employment in the control of schistosomiasis because of their relatively low cost.

The experiments in question were preliminary, and additional field trials are needed before final conclusions can be drawn as to the practicability of employing these compounds in the control of the disease. Additional work is needed to ascertain whether control of the snail intermediate host in an endemic area could be achieved with the treatment of all watersheds within the area. Long-term observations would have to be made in order to establish whether a complete kill is obtained and the time necessary for reintroduction of snails into the area. From the results obtained with sodium pentachlorophenate in the Los Peña stream, it would appear to the authors that a given watershed would not require re-treatment for a period of at least 6 months and perhaps not for 1 year. However, the question can only be settled by additional observations.

More data are needed on the minimum effective concentration of the chemicals for the destruction of the snail intermediate hosts. Opportunity was not afforded in the present work to establish these facts. A residual action exercised over a period of 6 weeks in two experiments gives some indication that the effect of the chemicals would probably persist for some time in stationary bodies of water. It seems improbable that any residual action could be expected in streams since the chemical would be carried off by the flow. Additional studies are needed on the effect of the most promising compounds on aquatic life. It is not known whether the chemicals would be destructive to the eggs of crayfish and various species of fish. In areas where fish are of economic importance, streams could be restocked after treatment, if, as seems likely, the aquatic environment is not disturbed to the point of being unfavorable for their maintenance. It seems likely that no permanent alterations occur in such environment. Twenty-four hours after the application of sodium pentachlorophenate to the stream at Los Peña, guppies had returned to the treated area. More information is needed on the toxicity of the compounds for mammals and further tests are being conducted at this time.

Summary

Eleven chemical compounds which proved effective in killing *Australorbis glabratus* in the laboratory in dilutions of 10 ppm or less were tested on this same species of snail in its natural environment in Puerto Rico. Six of these proved to be very effective molluscacides although the present price of four of them (2,4,6-triiodophenol, 2,4,6-triiodophenol sodium salt, 2,4,6-tribromophenol, and pentabromophenol) may prohibit their use on a large scale. Two compounds (sodium pentachlorophenate and copper pentachlorophenate) are excellent molluscacides and their cost is reasonable. In a stream near Los Peña, sodium pentachlorophenate at 9.5 ppm calculated on a 6-hour flow-rate dose destroyed all snails for a distance of 1½ miles downstream in spite of the entrance of three untreated tributary streams within this area. Embryos within the snail eggs were also killed. The compound was lethal to catfish, guppies, and eels, but apparently did not affect crayfish.

The toxicity of the effective compounds for mammals is under further study, and field trials are being extended to other areas.

ACKNOWLEDGMENTS

We acknowledge with sincere thanks the splendid laboratory facilities offered us by the Director of the School of Tropical Medicine of the University of Puerto Rico and also the help of assistants in the Department of Parasitology both in the laboratory and in the field, particularly the aid given by Thomas Raymond and Carlos Thillet, Jr. Nelson Biaggi accompanied the authors on several field trips and gave valuable assistance in the application of the chemical in flowing waters. The United States Geological Survey kindly lent the necessary field equipment for measuring the volume of water in flowing streams and were generous with their time in giving instructions on the use of the technical equipment. Mammalian toxicity tests were made on one particular compound by members of the staff of the Division of Animal Husbandry, Insular Experimental Station of the University of Puerto Rico. Automotive transportation was supplied by the Communicable Disease Center, Public Health Service, and particular thanks are extended to S. A. Sanitarian E. C. Kohler of that division for his suggestions and assistance. José Acosta Velarde, Chief, Land Authority of the Government of Puerto Rico, granted permission to apply the compounds in a large swamp, and his superintendent at Vega Baja, Carlos Javierri, fenced a large area to prevent cattle from drinking the treated water. To Dr. Juan A. Pons, Commissioner of Health, and to Dr. Guillermo Arbona, Chief, Division of Public Health, who gave permission to test the several compounds in various places in Puerto Rico, and to all others who aided in the experiments we are greatly indebted.

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The Visual Identification of V and W Form Colonies in *Salmonella* Cultures

By Maurice Landy, Ph. D.*

Shortly after the discovery of Vi antigen in typhoid cultures by Felix and Pitt in 1934 (*1*), Kauffmann introduced the terminology of V and W forms to describe certain colonial forms in *Salmonella* cultures containing Vi antigen. According to his terminology, the V form colony consists of organisms which possess Vi antigen and are relatively O-inagglutinable while organisms constituting the W form colony contain no Vi antigen and are readily agglutinated by O antiserum. Frequently, both forms occur in the same culture. Indeed, with certain organisms such as *Salmonella coli* 5396/38 (*2*) and *S. ballerup* we have been unable to isolate either form in the pure state. Kauffmann (*3*), Craigie and Brandon (*4*), and Giovanardi (*5*) observed that in cultures containing both V and W forms, these colonies were readily distinguishable when viewed by refracted light. The V form colony appeared opaque, whereas the W form was relatively translucent. This visual means of differentiating the V and W forms is a most practical and convenient laboratory procedure. A glance will

* Chief, Typhoid Research Section, Department of Biologic Products, Army Medical Department Research and Graduate School.

show whether certain *Salmonella* cultures are in the V or W state, or are a mixture of both forms.[1] The ease with which these colonial types may be differentiated by visual means is dependent largely on the optical arrangement for viewing with oblique illumination. Consequently, a very simple lighting arrangement employed in this laboratory, which has proved valuable for this purpose, is described.

The visual examination of V and W forms, as performed in this laboratory, follows. The mirror is removed from its attachment to a dissecting microscope base and placed concave side uppermost on the laboratory bench equidistant from the microscope lamp (with blue glass filter removed) and the dissecting microscope. A convenient distance between lamp and microscope is from 4 to 6 inches. The microscope lamp is tilted down so that the light strikes the center of the mirror and is deflected up through the glass stage of the dissecting microscope. While viewing a streak plate on the microscope stage, the proper intensity and centering of the light can be achieved easily by manipulating the mirror until maximum contrast is obtained. The magnification obtained with a 2× objective and 10× ocular is suitable for colony differentiation. When the light source is arranged as described, V and W forms (*S. typhosa*, *S. paratyphi C* (East Africa), *S. coli* and *S. ballerup*) exhibit a characteristic appearance. The V forms appear very dense and with the refracted light exhibit a reddish-coppery color, while the W forms are translucent and appear grayish-green. When both colonial forms are found in the same field, as frequently is the case, the contrast in appearance is immediately apparent. It has been observed that while the appearance of the W forms of typhoid, coli, and ballerup cultures is similar, the V forms of these cultures present a somewhat varied picture. The degree of density of the colony and the coppery appearance, or color, appears to be associated with the quantity of Vi antigen present. Thus, colonies of *S. coli* and *S. ballerup* cultures, which are a richer source of Vi antigen than is *S. typhosa*, invariably are more dense in appearance and exhibit a deeper copper color.

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[1] While the colonial appearance of V form colonies generally is characteristic, it still is advisable to verify the presence of Vi antigen by emulsification of one or more colonies and by a slide agglutination test with a pure Vi antiserum.

Incidence of Disease

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

UNITED STATES

Reports From States for Week Ended July 8, 1950

New cases of acute poliomyelitis reported in the Nation for the current week numbered 478, an increase over the 390 cases reported for the preceding week. However, the number was lower than the 683 cases reported for the corresponding week last year. The cumulative total (2,529) for the current "disease" year was below the corresponding total of 2,954 for the last year, the highest year on record. The "disease" year for acute poliomyelitis begins with the twelfth week of the calendar year.

For the current week, all geographic divisions except two showed increases over the preceding week in reported cases of acute poliomyelitis. These increases ranged from 1 in the New England States

Comparative Data for Cases of Specified Reportable Diseases: United States

[Numbers after diseases are International List numbers, 1948 revision]

Disease	Total for week ended—		5-year median 1945–49	Seasonal low week	Cumulative total since seasonal low week		5-year median 1944–45 through 1948–49	Cumulative total for calendar year		5-year median 1945–49
	July 8, 1950	July 9, 1949			1949–50	1948–49		1950	1949	
Anthrax (062)	1	-----	(1)	(1)	(1)	(1)	(1)	25	33	(1)
Diphtheria (055)	61	80	132	27th	7,399	8,882	13,863	3,128	3,768	6,297
Acute infectious encephalitis (082)	15	10	8	(1)	(1)	(1)	(1)	381	279	239
Influenza (480–483)	590	527	527	30th	275,231	110,982	180,460	244,701	74,712	136,902
Measles (085)	5,961	5,742	5,742	35th	290,499	627,006	561,860	271,369	574,613	526,914
Meningococcal meningitis (057.0)	66	54	54	37th	3,258	2,890	3,130	2,345	2,046	2,158
Pneumonia (490–493)	913	848	-----	(1)	(1)	(1)	-----	56,222	50,951	-----
Acute poliomyelitis (080)	478	683	311	11th	[2] 2,529	2,954	1,696	[2] 3,663	3,869	2,163
Rocky Mountain spotted fever (104)	24	20	20	(1)	(1)	(1)	(1)	193	258	193
Scarlet fever (050)	413	366	614	32d	55,169	78,970	86,258	38,730	56,426	59,572
Smallpox (084)	-----	-----	1	35th	43	50	195	23	40	141
Tularemia (059)	19	19	19	(1)	(1)	(1)	(1)	530	659	539
Typhoid and paratyphoid fever (040, 041)[3]	86	118	101	11th	998	981	1,087	1,508	1,469	1,560
Whooping cough (056)	2,504	1,295	1,648	39th	[4] 93,245	38,799	82,489	[4] 71,709	28,766	51,223

[1] Not computed.
[2] Deductions: Michigan, week ended Apr. 14, 1 case; Georgia, week ended July 1, 1 case.
[3] Including cases reported as salmonellosis.
[4] Addition: Indiana, week ended June 17, 20 cases.

to 37 in the South Atlantic States. Decreases from the same period were shown in the West South Central States (from 168 to 150) and the Mountain States (from 8 to 7). Texas reported the largest number of cases (105) but at the same time showed a decrease from the preceding week (124).

The total number of cases of influenza reported for the current week was 590, compared with 527 for the corresponding period last year. The cumulative total for the "disease" year was 275,231 cases of influenza. The 5-year (1945–49) median was 180,460.

Reported cases of meningococcal meningitis for the week numbered 66, compared with 72 for the preceding week, 54 for the corresponding week last year, and 54 for the 5-year median. The cumulative total for the current calendar year was 2,345, compared with the 5-year median of 2,158 cases.

The number of cases of acute infectious encephalitis reported for the week was 15, a decrease from the preceding week (22). For the corresponding week last year 10 cases were reported. The 5-year median for the week was 8 cases. The cumulative total of reported cases during the present calendar year was 381, which may be compared with the corresponding figure of 279 for 1949 and 239 for the 5-year median.

The total number of cases of whooping cough reported for the week in the Nation numbered 2,504, compared with 2,289 the preceding week, 1,295 for the corresponding week last year, and 1,648 for the 5-year median. The cumulative total for the current calendar year was 71,709, compared with 51,223 for the 5-year median.

No smallpox was reported in the United States. One case of anthrax was reported in Georgia, and one fatal case of bubonic plague was reported in San Miguel County, N. Mex.

Deaths During Week Ended July 8, 1950

	Week ended July 8, 1950	*Corresponding week, 1949*
Data for 92 large cities of the United States:		
Total deaths	8,401	9,267
Median for 3 prior years	8,892	----------
Total deaths, first 27 weeks of year	255,423	254,141
Deaths under 1 year of age	614	692
Median for 3 prior years	692	----------
Deaths under 1 year of age, first 27 weeks of year	16,653	17,420
Data from industrial insurance companies:		
Policies in force	69,703,883	70,327,350
Number of death claims	8,461	9,750
Death claims per 1,000 policies in force, annual rate	6.3	7.2
Death claims per 1,000 policies, first 27 weeks of year, annual rate	9.7	9.4

Reported Cases of Selected Communicable Diseases: United States, Week Ended July 8, 1950

[Numbers under diseases are International List numbers, 1948 revision]

Area	Diphtheria (055)	Encephalitis, infectious (082)	Influenza (480–483)	Measles (085)	Meningitis, meningococcal (057.0)	Pneumonia (490–493)	Poliomyelitis (080)
United States	**61**	**15**	**590**	**5,961**	**66**	**913**	**478**
New England	**4**			**611**	**2**	**24**	**8**
Maine	1			21		8	2
New Hampshire				1			
Vermont				33			1
Massachusetts	3			379	1		3
Rhode Island						1	1
Connecticut				177	1	15	1
Middle Atlantic	**4**	**6**	**1**	**2,021**	**10**	**262**	**51**
New York	4	5	(1)	732	5	166	29
New Jersey		1	1	976	2	53	16
Pennsylvania				313	3	43	6
East North Central	**8**	**1**	**4**	**1,783**	**13**	**110**	**40**
Ohio	3			346	5	19	7
Indiana	3			77		8	
Illinois	2	1	3	408	3	55	19
Michigan				388	3	21	8
Wisconsin			1	564	2	7	6
West North Central	**3**		**6**	**223**	**7**	**118**	**44**
Minnesota	1		2	32	2	4	7
Iowa	1			36			12
Missouri			1	67	4	16	1
North Dakota				9		90	1
South Dakota	1			21			1
Nebraska			3	34		3	13
Kansas				24	1	5	9
South Atlantic	8	1	144	**227**	6	54	84
Delaware				14			
Maryland			2	47		9	
District of Columbia				14	1	5	5
Virginia			136	61	1	18	22
West Virginia			2	33	1	3	5
North Carolina	4			17			4
South Carolina	1	1	1	8		1	31
Georgia	3		3	8	2	8	9
Florida				25	1	10	8
East South Central	**9**		**10**	**121**	**5**	**50**	**50**
Kentucky	1			46	1	1	9
Tennessee	4		6	44	4		13
Alabama	4		4	24		40	20
Mississippi				7		9	8
West South Central	**25**	**3**	**362**	**261**	**13**	**214**	**150**
Arkansas			14	51	1	8	13
Louisiana	3			11	2	30	6
Oklahoma	4	1	15	20	2	11	26
Texas	18	2	333	179	8	165	105
Mountain			**59**	**294**	**1**	**39**	**7**
Montana			26				1
Idaho				26	1		1
Wyoming				9			
Colorado			5	121		20	
New Mexico				6		3	3
Arizona			27	8		11	
Utah			1	124		5	2
Nevada							
Pacific		**4**	**4**	**420**	**9**	**42**	**44**
Washington				31	3	3	4
Oregon				6		15	6
California		4	4	383	6	24	34
Alaska						1	
Hawaii			1	5			

[1] New York City only.

Anthrax: Georgia, 1 case.

Reported Cases of Selected Communicable Diseases: United States, Week Ended July 8, 1950—Continued

[Numbers under diseases are International List numbers, 1948 revision]

Area	Rocky Mountain spotted fever (104)	Scarlet fever (050)	Smallpox (084)	Tularemia (059)	Typhoid and paratyphoid fever (040, 041)[1]	Whooping cough (056)	Rabies in animals
United States	**24**	**413**		**19**	**86**	**2,504**	**102**
New England		**36**				**184**	
Maine		3				20	
New Hampshire							
Vermont		1				16	
Massachusetts		27				93	
Rhode Island						3	
Connecticut		5				52	
Middle Atlantic	**5**	**84**			**12**	**297**	**14**
New York	3	[2] 42			6	108	14
New Jersey	1	13			2	99	
Pennsylvania	1	29			4	90	
East North Central		**159**			8	**586**	**27**
Ohio		108			4	213	11
Indiana		7				45	12
Illinois		12			1	62	3
Michigan		19			1	146	1
Wisconsin		13			2	120	
West North Central		**19**			**4**	**122**	**8**
Minnesota		4			1	18	
Iowa		1				31	8
Missouri		3			2	18	
North Dakota		2				8	
South Dakota							
Nebraska		4				2	
Kansas		5			1	45	
South Atlantic	**11**	**31**		**3**	**16**	**423**	**22**
Delaware						5	
Maryland	1	3			1	70	
District of Columbia						2	
Virginia	4	10			2	137	2
West Virginia		2			2	87	6
North Carolina	5	5			3	77	
South Carolina	1	3		1		23	7
Georgia		4		2	7	15	6
Florida		4			1	7	1
East South Central	**1**	**26**		**2**	**18**	**63**	**11**
Kentucky		4			15	18	6
Tennessee	1	13			1	9	
Alabama		4			2	29	5
Mississippi		5		2		7	
West South Central	**1**	**7**		**14**	**18**	**489**	**17**
Arkansas	1			14	2	33	1
Louisiana		2				4	
Oklahoma		1			3	47	1
Texas		4			13	405	15
Mountain	**4**	**9**			**3**	**131**	**1**
Montana					2	18	
Idaho						17	
Wyoming	1					4	
Colorado	3	2				30	1
New Mexico					1	6	
Arizona		5				37	
Utah		1				19	
Nevada		1					
Pacific	**2**	**42**			**7**	**209**	**2**
Washington		2				54	
Oregon	1	3			1	83	
California	1	37			6	72	2
Alaska							
Hawaii						2	

[1] Including cases reported as salmonellosis.
[2] Including cases reported as streptococcal sore throat.

FOREIGN REPORTS

CANADA

Reported Cases of Certain Diseases—Week Ended June 24, 1950

Disease	Newfoundland	Prince Edward Island	Nova Scotia	New Brunswick	Quebec	Ontario	Manitoba	Saskatchewan	Alberta	British Columbia	Total
Brucellosis							2				2
Chickenpox			8	4	89	228	36	8	50	149	572
Diphtheria					1						1
Dysentery, bacillary					1	2					3
Encephalitis, infectious						2					2
German measles			4	21	10	835	11	90	114	423	1,508
Influenza						6	7				13
Measles					256	723	20	20	26	224	1,269
Meningitis, meningococcal			1	1		4	1		4		11
Mumps			25	53	80	311	8	37	68	121	703
Poliomyelitis						2				2	4
Scarlet fever	3		1	2	47	15	3	8	29	10	118
Tuberculosis (all forms)	9		4	5	134	19	9	18	5	24	227
Typhoid and paratyphoid fever					13	3					16
Venereal diseases:											
Gonorrhea	17		9	11	84	51	20	15	47	56	310
Syphilis	2		1	5	47	18	13	3	3	11	103
Whooping cough	1		6	1	80	60	3	2	1	47	201

JAMAICA

Reported Cases of Certain Diseases—4 Weeks Ended June 24, 1950

Disease	Kingston	Other localities	Total	Disease	Kingston	Other localities	Total
Chickenpox	21	73	94	Paratyphoid fever		1	1
Diphtheria	1	2	3	Poliomyelitis		1	1
Dysentery, unspecified	2	3	5	Puerperal sepsis		2	2
Erysipelas	2	1	3	Tuberculosis, pulmonary	23	42	65
Leprosy	1	2	3	Typhoid fever	10	49	59
Meningitis, meningococcal	1	1	2	Typhus fever (murine)	5		5

NEW ZEALAND

Reported Cases of Certain Diseases and Deaths—4 Weeks Ended May 27, 1950

Disease	Cases	Deaths	Disease	Cases	Deaths
Brucellosis	5		Malaria	1	
Diphtheria	7		Meningitis, meningococcal	15	
Dysentery:			Poliomyelitis	6	1
Amebic	7		Puerperal fever	3	
Bacillary	23		Scarlet fever	81	
Encephalitis, infectious	3		Tetanus	8	4
Erysipelas	11		Tuberculosis (all forms)	138	34
Food poisoning	21	1	Typhoid fever	5	1

JAPAN

Reported Cases and Cumulative Totals of Certain Diseases and Deaths
5 weeks ended Apr. 29, 1950

Disease	5 weeks ended Apr. 29, 1950		Total reported for the year to date	
	Cases	Deaths	Cases	Deaths
Diarrhea, infectious	11		21	
Diphtheria	1,215	110	4,797	507
Dysentery, unspecified	828	130	1,851	382
Filariasis	6		32	
Gonorrhea	17,032		53,792	
Influenza	812		16,032	
Leprosy	75		190	
Malaria	54	2	192	18
Measles	8,353		21,559	
Meningitis, meningococcal	134	36	407	97
Paratyphoid fever	73	5	270	13
Poliomyelitis	130		494	
Pneumonia	20,291		79,612	
Puerperal infection	87		302	
Rabies	9		25	
Scarlet fever	474	4	1,440	9
Schistosomiasis	43		115	
Smallpox	1		4	
Syphilis	13,284		43,419	
Tetanus	170		494	
Tuberculosis	46,880		135,448	
Trachoma	13,018		39,386	
Typhoid fever	311	44	1,013	154
Typhus fever	95	6	766	49
Whooping cough	12,324		43,520	

4 weeks ended May 27, 1950

Disease	4 weeks ended May 27, 1950		Total reported for the year to date	
	Cases	Deaths	Cases	Deaths
Diarrhea, infectious	3		25	
Diphtheria	807	50	5,596	556
Dysentery, unspecified	1,826	290	3,674	672
Encephalitis, Japanese "B"	1		1	
Filariasis	6		38	
Gonorrhea	13,520		67,493	
Influenza	505		16,768	
Leprosy	54		244	
Malaria	69	2	261	20
Measles	8,771		30,320	
Meningitis, meningococcal	67	28	473	125
Paratyphoid fever	160	12	433	25
Poliomyelitis	150		644	
Pneumonia	11,386		91,001	
Puerperal infection	59		361	
Rabies	2		23	
Scarlet fever	633	3	2,076	12
Schistosomiasis	54		169	
Smallpox			3	
Syphilis	9,890		53,306	
Tetanus	154		650	
Tuberculosis	37,617		173,066	
Trachoma	20,074		59,456	
Typhoid fever	451	46	1,462	199
Typhus fever	64	2	829	51
Whooping cough	10,529		54,049	

WORLD DISTRIBUTION OF CHOLERA, PLAGUE, SMALLPOX, TYPHUS FEVER, AND YELLOW FEVER

The following tables are not complete or final for the list of countries included or the figures given. Since many of the figures are from weekly reports, the accumulated totals are for approximate dates.

CHOLERA

(Cases)

Place	January–April 1950	May 1950	June 1950—week ended—			
			3	10	17	24
ASIA						
Burma	8	7		2	1	
Akyab		1		1		
Bassein	1			1	1	
Maubin	3					
Rangoon	1					
India	45,035	13,177	317	320	297	272
Ahmedabad		7				
Allahabad		3				
Bombay		[1] 1		1		
Calcutta	[2] 4,797	1,712	316	318	283	260
Cawnpore		1				
Cocanada	2					
Cuddalore	31					
Lucknow		3	1	1	4	
Madras	11					
Masulipatam	46					
Negapatam	67					
New Delhi						12
Port Blair (Andaman Island)	[1] 2					
Tellicherry	27					
Tuticorin	25					
Indochina (French)	6	6			1	
Cambodia	5					
Cochinchina	1	6			1	
Giadinh		3				
Rachgia	1					
Pakistan	15,176	3,944	[3] 370	[3] 47	[3] 47	
Chittagong	53	72	14	10	9	
Dacca	153	29	4			

[1] Imported. [2] Includes imported cases. [3] Preliminary figures.

PLAGUE

(Cases)

AFRICA						
Belgian Congo	4	3	1		1	1
Costermansville Province	2	2				
Stanleyville Province	2	1	1		1	1
Madagascar	41	3			[1] 1	
Rhodesia, Northern	2					
Union of South Africa	8	1				
Orange Free State	6	1				
ASIA						
Burma	[2] 204	4	2	1		
Bassein	1					
Bhamo	[3] 2		1			
Henzada	12					
Kyaiklat	34					
Moulmein		[3] 1	1			
Myaungmya	5					
Myingyan	2					
Pegu	1					
Pyapon	1					
Rangoon	[2] 2	[2] 3		1		
Yenangyaung	58					
China:						
Chekiang Province	10					
Wenchow	[4] 4					
Fukien Province	[5] 121					
Kwangsi Province	[4] 63					
Kwangtung Province	15					

See footnotes at end of table.

Place	January–April 1950	May 1950	June 1950—week ended— 3	10	17	24
ASIA—continued						
India	35,920	[6] 582				
Allahabad	[3] 14	[3] 4	[3] 1			
Bombay	2	[2] 3				
Calcutta	[3] 1	2				
Cawnpore	18					
Lucknow	[2] 8	[3] 1				
Indochina (French):						
Annam	42	16	2	2	3	5
Phanthiet	38	16	2	2	3	5
Cambodia	9	[7] 36		1		
Pnompenh	3					
Cochinchina	2	5				
Laos	2					
Indonesia:						
Java	293	26				
Bandoeng	2					
Jogjakarta	[8] 132	26	7	4	3	
Pakistan	[3] 1					
Karachi	[3] 1					
Thailand (Siam)	52	4				
SOUTH AMERICA						
Ecuador	10	2				
Chimborazo Province		2				
El Oro Province	4					
Loja Province	6					
Peru	6	3				
Lambayeque Department		1				
Lima Department	1					
Piura Department	5	2				
Venezuela	5					
Miranda State	5					

[1] June 11–20, 1950. [2] Includes imported cases. [3] Imported. [4] Deaths.
[5] Corrected figure. Includes 9 deaths reported as cases on Kinmen Island.
[6] Preliminary figures. [7] Includes suspected cases. [8] Corrected figure.

SMALLPOX

(Cases)

AFRICA						
Algeria	43	30		[1] 2		
Angola	80					
Bechuanaland	7					
Belgian Congo	967	293	87	44		
British East Africa:						
Kenya	10					
Nyasaland	226	11		[2] 2	1	
Tanganyika	366	126	2		2	8
Uganda	1	1				
Cameroon (British)	233	5				
Cameroon (French)	34	54		[2] 3		
Dahomey	182	15		[2] 2		
Egypt	[3] 4					
Eritrea	1					
Ethiopia	7					
French Equatorial Africa	403	14				
French Guinea	5	6		[2] 1		
French West Africa: Haute Volta	127	45		[2] 16		
Gambia	4	1				
Gold Coast	33	61	4		[4] 3	
Ivory Coast	444	49				
Libya	2					
Mauritania	1					
Morocco (French)	5					
Mozambique	89	18				
Nigeria	10,150	1,280	[5] 8	[5] 10	[5] 10	
Niger Territory	635	260		[2] 29		
Rhodesia:						
Northern	4					
Southern	275	55				
Senegal	2					
Sierra Leone	25					

See footnotes at end of table.

Place	January–April 1950	May 1950	June 1950—week ended— 3	10	17	24
AFRICA—continued						
Sudan (Anglo-Egyptian)	48	4	1		19	
Sudan (French)	93	3				
Togo (French)	42				[4] 6	
Tunisia	1					
Union of South Africa	283	[6] 4			[6] 1	
ASIA						
Afghanistan	215	40				
Arabia	320					
Bahrein Islands: Bahrein	34					
Burma	4,798	110	15	12	8	
China	624					
India	69,365	18,329	[7] 274	[7] 197	[7] 186	[7] 127
India (Portuguese)	19	1				
Indochina (French)	264	8	2	5	1	1
Indonesia:						
Borneo	112	32	1	6	51	
Java	948	717	184	194	210	
Sumatra	188	44	17	8	1	
Iran	148	19	4	2		
Iraq	100	14	1	10	1	3
Israel	15					
Japan	4					
Korea (Republic of)	1,271	[7] 47				
Lebanon	[8] 1	1				
Netherlands New Guinea	3					
Pakistan	8,353	2,775	474	244	113	
Palestine	87					
Syria	15					
Thailand (Siam)	457	3				
Transjordan	27	2			1	
Turkey (See Turkey in Europe.)						
EUROPE						
Great Britain:						
England: Liverpool	[8] 1					
Scotland: Glasgow	21					
Greece		1		2		12
Athens				1		
Piraeus						1
Xylokastron		1				
Turkey	8					
NORTH AMERICA						
Guatemala	2					
Mexico	111	22	4		13	
SOUTH AMERICA						
Argentina	353	45				
Brazil	27		1	1		
Chile	2,847	635		41		45
Colombia	499	12				
Ecuador	76	7				
Paraguay		1				
Peru	510					
Venezuela	42					
OCEANIA						
Australia: Fremantle	[8] 1					

[1] In Algiers. [2] June 1–10, 1950. [3] Includes imported cases. [4] June 11–20, 1950. [5] In Lagos only. [6] In Johannesburg only. [7] In ports only. [8] Imported.

TYPHUS FEVER*

(Cases)

AFRICA						
Algeria	63	20		[1] 1		
Basutoland	20	2				
Belgian Congo	[2] 39	4				
British East Africa: Kenya	[2] 7					
Egypt	40	24	1	4	5	2
Eritrea	8	2		1	2	1

See footnotes at end of table.

Place	January–April 1950	May 1950	June 1950—week ended—			
			3	10	17	24
AFRICA—continued						
Ethiopia	213					
French Equatorial Africa	4					
Gold Coast	6					
Libya	67	16	1	2	3	1
Madagascar	[3] 1			[1] 1		
Morocco (French)	3	1				
Morocco (International Zone)	1					
Morocco (Spanish Zone)	3					
Nigeria	1					
Rhodesia, Southern	[4] 5					
Sierra Leone	[3] 1					
Sudan (Anglo-Egyptian)	4					
Tunisia	38	11				
Union of South Africa	[2] 30	[3] 1				
ASIA						
Afghanistan	929	248				
Burma	[3] 8					
China	[2] 8					
India	182	55				
India (Portuguese)	8					
Indochina (French)	12	18				
Indonesia:						
Java	6					
Sumatra	1					
Iran	115	18	6			
Iraq	79	11	7		7	7
Japan	766	27	1	2	3	2
Korea (Republic of)	[3] 1,103	[3] [5] 58				
Lebanon	1					
Pakistan	54	14	2			
Palestine: Jerusalem		1				
Straits Settlements: Singapore	[2] 3	[3] 1				
Syria	[2] 31	4			2	
Transjordan	12	1				1
Turkey (see Turkey in Europe).						
EUROPE						
France	1					
Germany (British Zone)	1					
Germany (French Zone)	2					
Germany (United States Zone)	1					
Great Britain:						
England: Liverpool				[3] [6] 1		
Island of Malta	[3] 2	[3] 1				
Greece	[2] 20					
Hungary	3					
Italy	24	57				
Sicily	16	57				
Poland	37					
Spain	12	1	1			
Turkey	110	22	10		8	
Yugoslavia	138	71				
NORTH AMERICA						
Costa Rica [3]	3					
Guatemala	9					
Jamaica [3]	7	4	5			
Mexico [3]	38	6	1	2	3	1
Panama Canal Zone	[3] 1					
Puerto Rico [3]	9					
SOUTH AMERICA						
Argentina	[2] 2					
Chile	52	11	1	3	2	
Colombia	[2] 332	[2] 22				
Curacao	1					
Ecuador	[2] 77	[2] 8				
Peru	[7] 251					
Venezuela	[3] 5	[3] 1				
OCEANIA						
Australia [3]	57	7	7			
Hawaii Territory	[3] 2	[3] 1				

*Reports from some areas are probably murine type, while others include both murine and louse-borne types.

[1] June 1–10, 1950. [2] Includes murine type. [3] Murine type. [4] April 1–30, 1950.
[5] In Inchon and Seoul. [6] Imported. [7] Jan. 1–Feb. 28, 1950. [8] Off-shipping.

YELLOW FEVER

(C=cases; D=deaths)

Place		January–April 1950	May 1950	June 1950–week ended—			
				3	10	17	24
AFRICA							
French Equatorial Africa	C	1					
Port Gentil	C	[1] 1					
Gold Coast	C	10					
Ankobra Ferry	D	1					
Kade	C	1					
Oda Area:							
Akwatia	C	[2] 7					
Atiankama	C	1					
Sierra Leone	C	1			[1] 1		
Koinadugu District	C	1			1		
NORTH AMERICA							
Panama:							
Colon	D	1					
SOUTH AMERICA							
Bolivia:							
Chuquisaca Department	C	[3] 850					
La Paz Department	C		[4] 17				
Colombia:							
Mocoa Locality	D	2					
Peru:							
Cuzco Department	D	[5] 2					
Quincemil	D	2					
Huanuco Department	C	[6] 1					
Tingo Maria	C	1					
Junin Department	D				1		
San Ramon	D				1		
San Martin Department	D	1	1				
Juanjui	D	[5] 1					
Lamas	D		1				

[1] Suspected. [2] Includes 4 suspected cases. [3] Reported in Azero Province during the period Jan. 1–Mar. 14, 1950, with 230 deaths. [4] Outbreak in North and South Yungas Provinces. Eight deaths reported. [5] February 1950. [6] April 1950.

Bubonic Plague in San Miguel County, N. Mex.

Under date of July 11, 1950, positive cultures of bubonic plague were reported in Pecos, San Miguel County, N. Mex., by the State Health Department. An autopsy was performed July 4, the day that the patient died, but no gross signs of plague were observed. Cultures were sent to the Western Communicable Disease Center Laboratory, San Francisco, for further study.