POTENTIAL INFECTIOUS HAZARDS OF LABORATORY TECHNIQUES

III. Viral Techniques[1]

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Received for publication April 23, 1954

Workers engaged in viral or rickettsial investigations are well aware of the numerous laboratory infections that plague them. In the literature are reports of laboratory infections with, to name a few, Psittacosis, Poliomyelitis, Hepatitis, Equine Encephalomyelitis, Q fever, Epidemic Typhus, Scrub Typhus, and Rocky Mountain Spotted Fever (Sulkin and Pike, 1951). Each laboratory has set up its own standard operating procedures (Smadel, 1951). Unfortunately, the standards of safety are usually limited at some points by the funds available for equipment. As a result, in some laboratories and with some organisms, it is a foregone conclusion that sooner or later many of the personnel would become infected with the agent studied. Realization of this fact is a deterrent to otherwise desirable research. Many laboratories rely heavily on a strong immunization program. Immunization is desirable when available, but better protection of the laboratory worker is achieved when immunization is combined with a policy of containment of the organisms. The first step in this direction is a determination of where and how the infectious particles gain access to the environment. This paper is the third in a series of papers devoted to studies on infectious hazards of laboratory techniques (Reitman *et al.*, 1954*a*,*b*).

An investigation has been made of the aerosols produced and of the extent of contamination during the following viral and rickettsial techniques: (1) diluting viral particles in vaccine bottles, (2) mouse inoculation via the intranasal and intracerebral routes, (3) egg inoculation via the allantoic and yolk sac routes, (4) inoculation of the chorioallantoic membrane by the window technique, (5) harvesting allantoic and amniotic fluid, and (6) the maceration of mouse brain tissue in the Ten Broeck grinder.

[1] Presented in part at the 53rd General Meeting of the Society of American Bacteriologists held in San Francisco, Calif., August 10–14, 1953.

Coliphage T-3 was used as the test organism. Air and surface samples were taken by means of sieve type air samplers (DuBuy and Crisp, 1944) and cotton swabs, respectively. The sampling medium used was tryptose-phosphate-glucose agar for the sieve sampler plates and tryptose-phosphate-glucose broth for the cotton swabs. Air samples were taken at the rate of one cu ft per minute. These plates were then covered with a layer of *Escherichia coli*, strain B, seeded tryptose-phosphate-glucose agar (0.7 per cent agar) and incubated 18 to 24 hours at 37 C at which time plaque counts were made. Surface samples taken by cotton swabs moistened with nutrient broth were immersed and shaken in melted tryptose-phosphate-glucose agar which was then seeded with *E. coli*, strain B, and poured into tryptose-phosphate-glucose agar plates.

The operational area was surrounded by sieve samplers (figure 1) by placing 3 samplers on the table and suspending 4 samplers 8 to 12 inches above the table. Control air and surface samples were taken before the beginning of each operation.

VACCINE BOTTLE DILUTION METHOD

In the first experiment 9 tenfold dilutions of a phage suspension containing approximately 2.6×10^9 infectious particles per ml were made in vaccine bottles containing 9 ml of tryptose-phosphate-glucose broth. Two ml tuberculin luer-lok syringes equipped with 23 gauge three-quarter inch needles were used to transfer one ml amounts of the diluted suspension from one bottle to another. A fresh syringe and needle were used for each dilution. In one series the rubber diaphragms were swabbed with 70 per cent ethanol before making the dilutions, while the other series in addition to the initial ethanol swabbing the needle was surrounded by an ethanol soaked cotton pledget which served the twofold purpose of surrounding the point of

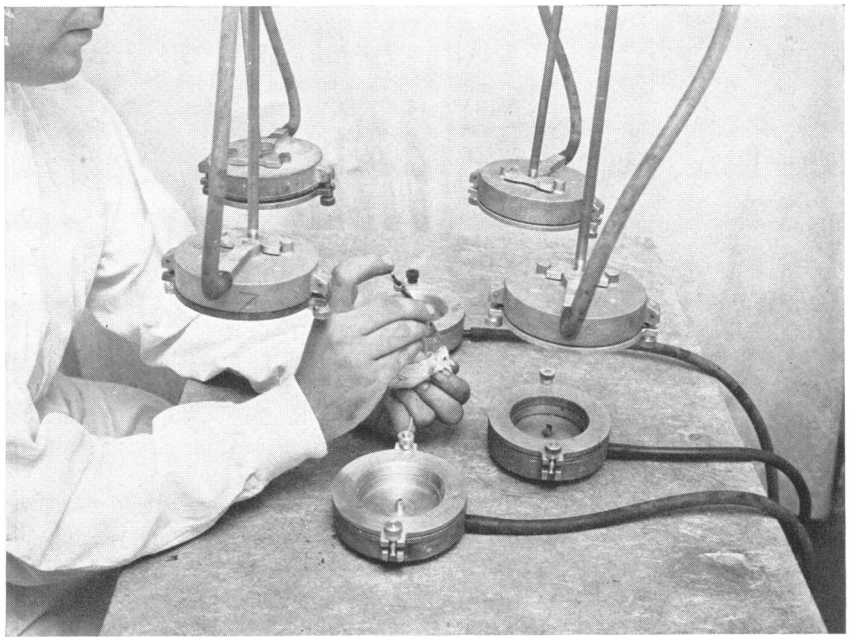

Figure 1. Air sampling by means of sieve type air samplers during intranasal inoculation of mice with T-3 coliphage.

withdrawal and of swabbing the diaphragm after the needle was removed.

Table 1 shows that when no attempt was made to surround the needle with a pledget, a small amount of aerosol was produced, as shown by air sampling, in 6 out of 10 tests. The number of plaques obtained ranged from 10 to 1. The use of an ethanol soaked pledget entirely eliminated the aerosol production.

Table 2 shows that dilutions in which contaminated diaphragms occurred ranged from 10^{-9} to 10^{-3} when no pledget was used, and 56 out of 90 diaphragms were contaminated with the test viruses. The pledget reduced diaphragm

TABLE 1

T-3 coliphage recovered from the air during and after making tenfold dilutions of phage suspensions in vaccine bottles*

Number of Tests	Total Number of Plaques	
	No cotton pledget around needle	Ethanol cotton pledget used
1	10	0
1	9	0
1	3	0
3	1	0
4	0	0

* Rubber diaphragm of the vaccine bottle was swabbed with 70 per cent ethanol before the needle was inserted.

All controls were negative for the test virus.

TABLE 2

T-3 coliphage recovered from rubber diaphragm after making tenfold dilutions of phage suspensions in vaccine bottles*

Number of Tests	Highest Dilution in Which Contaminated Diaphragm Found		
	No cotton pledget around needle	Number of tests	Ethanol cotton pledget used
1	10^{-9}	1	10^{-4}
2	10^{-7}	3	10^{-2}
1	10^{-6}	3	10^{-1}
4	10^{-5}	3	0
1	10^{-4}		
1	10^{-3}		
Total Number of Contaminated Diaphragms			
	56†		11†

* Rubber diaphragm of the vaccine bottle was swabbed with 70 per cent ethanol before the needle was inserted.

† Out of 90.

All controls were negative for the test virus.

contamination to 11 out of 90, and no diaphragm was found to be contaminated beyond the 10^{-4} dilution.

ANIMAL INOCULATION

Intranasal route. In the next experiment 10 mice were anesthetized with ether and inoculated intranasally with 0.05 ml of coliphage T-3. A series of 10 tests was done, each test using increasing 10-fold dilutions of phage. Table 3 shows a summary of the results obtained. The extent of aerosol production was directly proportional to the concentration of the inoculum. Starting off with undiluted phage suspension, each mouse receiving an inoculum of approximately 1.5×10^8 phage particles, a large amount of aerosol was produced which decreased sharply with each 10-fold dilution until it was practically negligible at the 10^{-5} dilution level (inoculum 1.5×10^3). Hand and table contamination was much greater than air contamination. Since the mouse was held in the operators' hand, and it in turn rested on the table, these areas received the brunt of aerosol contamination and any of the drippings that may have inadvertently contaminated the mouse fur.

Intracerebral inoculation. In the next experiment 10 mice were anesthetized with ether and inoculated intracerebrally with 0.03 ml of a phage suspension by means of a 27 gauge one-quarter inch needle. Each mouse received approximately 3.3×10^7 particles. In series A the injection site was swabbed with 70 per cent ethanol prior to injection; in series B the injection site was swabbed with ethanol before and after injection, and the needle was surrounded by an ethanol soaked cotton pledget; and in series C the injection site was swabbed with ethanol before injection, with two per cent tincture of iodine after injection, and the needle was surrounded by an ethanol soaked pledget.

TABLE 3

Summary of aerosols produced during inoculation of mice with varying amounts of T-3 coliphage via the intranasal route

Number of Phage Particles Inoculated per Mouse*	Average Number of Phage Particles Recovered Per Test as Shown by Plaque Counts Obtained by Means of Sieve Type Air Samplers	Extent of Contamination as Shown by Means of Cotton Swabs	
		Hand	Table
1.5×10^8	270	heavy	heavy
1.5×10^7	19.9	heavy	heavy
1.5×10^6	8.6	heavy	heavy
1.5×10^5	2.4	moderate	moderate
1.5×10^4	0.5	light	light
1.5×10^3	0.1	trace	trace

* For each dilution, 10 tests were made with 10 mice per test.

TABLE 4

Recovery of T-3 coliphage during and after intracerebral inoculation of mice with 3.3×10^7 particles

Series	Sieve Type Air Samples	Surface Samples with Cotton Swabs		
		Site of injection	Technicians' hands	Table
	Average no. of plaques per 10 mice	No. contaminated / No. injected	No. contaminated / No. tests*	No. contaminated / No. tests*
A	1.1	100/100	4/10	6/10
B	0.2	53/100	3/10	2/10
C	0.3	6/100	1/10	2/10

A—Inoculation site swabbed with 70 per cent ethanol before inoculation.

B—Inoculation site swabbed with 70 per cent ethanol before and after inoculation. Needle surrounded by 70 per cent ethanol soaked cotton pledget.

C—Inoculation site swabbed by 70 per cent ethanol before inoculation, swabbed with 2 per cent tincture of iodine after inoculation. Needle surrounded by 70 per cent ethanol soaked pledget.

* Ten mice per test.

Table 4 shows that the intracerebral inoculation of 100 mice produced only slight aerosols as shown by recovery of viral particles by the sieve samplers. The average recovery of phage per each 10 mice injected was 1.1 in series A, 0.2 in series B, and 0.3 in series C. However, the site of inoculation was grossly contaminated in 100 per cent of the animals injected when no attempt was made to decontaminate the inoculation site. This was reduced to 53 per cent when 70 per cent ethanol was used to decontaminate the site and the needle was surrounded by an ethanol pledget. The use of 2 per cent tincture of iodine together with an ethanol pledget reduced contamination to 6 per cent. The extent of contamination of the technician's hand and the

table top likewise was reduced by the use of the disinfectants and ethanol pledget.

EGG INOCULATION

Allantoic and yolk sac inoculation. In the next experiment 12 day old embryonated eggs were inoculated in the allantoic sac and in the yolk sac. Shells were swabbed with 2 per cent tincture of iodine before inoculation, and 0.25 ml of phage was inoculated into the allantoic sac with a 23 gauge three-quarter inch needle. The inoculation hole was then sealed with Duco cement. Surface samples were taken of the egg shell, technicians' hands, and egg tray. Ten eggs were inoculated per test. In the 10 tests that were made, phage was recovered in the air samples in very small amounts in only 2 tests. Only 4 out of 100 egg shells were contaminated. Phage was recovered from the technicians' hands in large amounts after 2 tests, and no recoveries were made from the tray.

Yolk sac inoculation was accomplished by introducing 0.25 ml of phage suspension with a 23 gauge 1½ inch needle through the air sac of iodine swabbed eggs. Egg shells were sealed as described before. Table 5 shows that as in the allantoic method only a slight aerosol was produced as judged by air sampling. Only 4 plaques appeared on the plates from one test. However, shell contamination was considerable.

When this experiment was repeated using an ethanol pledget around the needle, only 1 plaque was obtained on the air sampling plates and only 4 out of 100 eggs were contaminated.

Chorioallantoic membrane inoculation. An inoculum of 0.05 ml containing 1×10^9/ml was dropped onto the chorioallantoic membrane, and the opening was then sealed with scotch tape.

TABLE 5

Recovery of T-3 coliphage from egg shells after allantoic and yolk sac inoculation with T-3

Number of Eggs Found to be Contaminated* After		
Allantoic sac inoculation	Yolk sac inoculation	
	Without ethanol pledget	With ethanol pledget
4/100†	23/100	4/100

* By means of cotton swabs.

† $\frac{\text{Number contaminated}}{\text{Number inoculated}}$.

A total of 100 eggs were inoculated, 10 per test. No recoveries of phage were made on air sampler plates. Swabbing of egg shells revealed heavy contamination of the shell area around the window in two instances and light contamination in one. There was no contamination of the operator's hand or the egg tray in which the eggs were held during inoculation.

HARVESTING

Allantoic fluid. Embryonated eggs were inoculated with 0.5 ml of a 1×10^9/ml phage suspension; the shell was decontaminated with 70 per cent ethanol, chilled for two hours, and then harvested. Five eggs were harvested for each test. Air was sampled for 10 minutes. The allantoic fluid was removed via the air sac by aspirating with a 13 gauge needle and 5 ml syringe. Results are shown in table 6. A total of 57 phage particles was recovered on the air sampling plates during the harvesting of 50 eggs. The egg shells and egg trays were found to be heavily contaminated with the test virus. Fifty-three phage particles were recovered from the hands of the operator.

Amniotic fluid. Embryonated eggs were inoculated through the air sac with 0.5 ml of a 1×10^9/ml phage suspension by means of a 1½ inch needle. The shell was decontaminated with 70 per cent ethanol, chilled two hours, and

TABLE 6

Phage particles recovered during harvesting of allantoic fluid

Test No.*	Number of Phage Particles Recovered on Air Sampling Plates	Recovery of Phage Particles from Surfaces†			Phage Count Per Ml‡
		Hand	Egg tray	Shell	
1	11	0	1	TNTC	5.5×10^7
2	0	0	TNTC	TNTC	1.7×10^7
3	24	22	TNTC	TNTC	3.9×10^7
4	1	0	54	127	2.9×10^7
5	8	18	TNTC	TNTC	2.2×10^7
6	3	2	TNTC	TNTC	2.4×10^8
7	0	0	22	TNTC	1.0×10^8
8	3	6	0	TNTC	2.1×10^8
9	5	3	TNTC	TNTC	1.7×10^8
10	2	2	TNTC	TNTC	2.3×10^8

* Five harvestings per test.

† One swab was used per test.

‡ Count of pooled allantoic fluid from 5 eggs.

TNTC—Too numerous to count.

TABLE 7

Phage particles recovered during harvesting of amniotic fluid

Test No.*	Number of Phage Particles Recovered on Air Sampling Plates	Recovery of Phage Particles from Surfaces†			Phage Count Per Ml‡
		Hand	Egg tray	Shell	
1	0	TNTC	TNTC	TNTC	1.1×10^8
2	0	0	TNTC	TNTC	8.6×10^7
3	0	3	TNTC	TNTC	2.3×10^8
4	0	3	TNTC	TNTC	2.1×10^8
5	7	2	TNTC	TNTC	1.4×10^8
6	0	0	43	TNTC	4.1×10^8
7	0	0	TNTC	TNTC	3.7×10^8
8	1	0	TNTC	8	5.5×10^8
9	0	0	TNTC	TNTC	1.6×10^8
10	2	TNTC	TNTC	TNTC	6.0×10^7

* Five harvestings per test.
† One swab was used per test.
‡ Count of pooled amniotic fluid from 5 eggs.
TNTC—Too numerous to count.

then harvested. Five eggs were harvested by removing the shell over the air sac, cutting the shell membrane, decanting the allantoic fluid into 70 per cent ethanol, pulling up the amniotic membrane with forceps, and aspirating the amniotic fluid with a 13 gauge inch needle and 5 ml syringe. Results are shown in table 7. A total of 10 phage particles was recovered by means of air sampling in the 10 tests. The egg trays and egg shells were heavily contaminated with the test virus while the hands of the operator were found to be heavily contaminated in 2 out of 10 tests.

TABLE 8

Recovery of phage particles during the maceration of mouse brain tissue in a Ten Broeck grinder

Test No.	Number of Phage Particles Recovered on Air Sampling Plates	Recovery of Phage Particles from Surfaces*		
		Hand	Table	Plunger handle of grinder
1	1	0	0	4
2	2	0	0	TNTC
3	29	0	0	0
4	14	0	0	74
5	3	0	0	0
6	1	0	0	53
7	4	0	0	0
8	9	3	0	0
9	9	0	0	93
10	11	5	0	18

* One swab was used per test.
TNTC—Too numerous to count.

Maceration of mouse brain tissue. One normal mouse brain was placed into the bottom of a Ten Broeck grinder, and 0.25 ml of a 2.5×10^8/ml phage suspension was added. The tissue was then ground for 3 minutes. The average phage count of the homogenized tissue was 3.3×10^7/g. Table 8 shows that a total of 83 phage particles was recovered by air sampling during the maceration of 10 mouse brains. The plunger handle was found to be contaminated in 6 out of 10 tests while the operator's hands were contaminated in 2 tests.

DISCUSSION

The inherent hazard in the use of vaccine bottles for making dilutions of infectious materials lies in the contamination of the rubber diaphragm. This contamination was greatly reduced by the use of an ethanol soaked cotton pledget around the needle and by swabbing the diaphragm with alcohol. The production of slight aerosols by the vaccine bottle method was completely eliminated by the use of the ethanol pledget around the needle.

Intranasal inoculation of mice produced large amounts of aerosols together with extensive contamination of the environment. The only solution to this problem would seem to lie in the use of a ventilated cabinet to contain the organism and the use of ventilated cages to house the inoculated animals. The safest method for intracerebral inoculation involved the use of an ethanol soaked pledget around the needle together with decontamination of the injection site with two per cent tincture of iodine.

Allantoic and yolk sac inoculation of embryonated eggs produced only slight aerosols, but shell contamination by the latter method was considerable. This could be quite a problem when egg incubators equipped with fans are used. Shell contamination was greatly reduced by the use of an ethanol soaked pledget around the needle.

Inoculation of the chorioallantoic membrane by the window technique appears to be a relatively safe procedure except for the possibility of shell contamination which occurred in two instances.

Harvesting of infected allantoic and amniotic fluids appears to be a hazardous procedure. There was heavy surface contamination of the egg trays, shells, and hands of the operator.

Maceration of tissue in the Ten Broeck grinder produced aerosols of the test virus. Surface contamination was confined to the plunger handle.

The results of these experiments indicate the need of carrying out viral techniques in a protective ventilated cabinet (Wedum, 1953).

SUMMARY

The vaccine bottle method of making dilutions of virus produced slight aerosols which were eliminated by surrounding the syringe needle with a 70 per cent ethanol soaked cotton pledget. Fifty-six out of 90 rubber diaphragms were found to be contaminated with the test virus, coliphage T-3. Intranasal inoculation of mice is an extremely hazardous procedure. Large amounts of aerosols were produced by this technique. Intracerebral inoculation of mice produced surface contamination of the injection site. Insignificant aerosols were produced by allantoic, yolk sac, and chorioallantoic (window technique) inoculation of embryonated eggs, but shell contamination by the former two techniques was considerable. Harvesting of infected egg fluids produced extensive contamination of the working area.

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