A Study of the Stability of Pertussis Vaccine Under Different Conditions of Storage

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Stability and antigenic potency of any prophylactic agent become matters of concern to all who are involved in communicable disease control.

Early in our experience with pertussis vaccine we recognized certain problems in relation to stability of the product. A question of great practical importance was how long a particular lot of pertussis vaccine would remain antigenic under ordinary conditions of storage in the cold. Another practical question was whether it would be possible to prepare a reference vaccine which would retain its potency over a long period, for example, one in the lyophilized state. Also, the influence of certain killing agents and preservatives on the stability of pertussis vaccine was not known. The choice of a chemical such as merthiolate or phenol, instead of heat, had been made without experimental basis, in an attempt to use a killing agent that might be less likely to injure the undefined protective antigen. After chemically killed vaccines of pertussis cultures grown on Bordet-Gengou medium had been found, on the basis of field trials to be effective, there was a natural hesitancy to change the method of preparation in any way. However, the development of a mouse protection test provided laboratory criteria for assessing various factors concerned with antigenicity and stability. This report concerns particularly the results of mouse protection tests of vaccines stored at different temperatures for varying periods. Data are included also on the stability of vaccines killed and preserved by different agents, and of one vaccine prepared from growth in fluid medium (Cohen-Wheeler) for comparison with vaccines prepared from growth on solid medium (Bordet-Gengou).

Experimental Methods and Results

The experimental lots of pertussis vaccines were prepared from one culture, strain number 10-536, grown either on Bordet-Gengou medium or in the fluid medium described by Cohen and Wheeler.[1] Vaccines prepared from growth on Bordet-Gengou medium were harvested after 48–72 hours incubation, washed once in saline, and killed and preserved by one of the following agents: merthiolate 1:5,000 in a heavy bacterial suspension, subsequently diluted to con-

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Table 1—Pertussis Vaccine Lot 213: Mouse Protective Properties of Portions Killed by Merthiolate, Phenol, Formalin, and Heat, Respectively, After Storage in the Cold (5°–10°C)

Storage Period	Vaccine Lot 213 from Bordet-Gengou Medium								Recently Prepared Vaccine ED/50 (Reference)
	Merthiolate		Phenol		Formalin		Heat		
	ED/50	Ratio	ED/50	Ratio	ED/50	Ratio	ED/50	Ratio	
2 years 5 months	240	...	150	...	500	...	280	...	...
6 years 7 months	500	0.8	390	0.6	NG	...	400	0.6	620
7 years 6 months	140	0.9	290	1.8	250	1.6	230	1.4	160
8 years 3 months	320	3.2	430	4.3	310	3.1	190	1.9	100
9 years 8 months	690	4.6	690	4.6	NG	...	250	1.7	150
9 years 11 months	630	2.7	1,300	5.7	1,500	6.5	710	3.1	230

NG = response not graded
Ratio = ED/50 of test vaccine divided by ED/50 of reference

tain 10 billion organisms per ml and merthiolate 1:10,000; phenol, 0.5 per cent; formalin, 0.2 per cent; or heat at 56° C. for one hour, with merthiolate 1:10,000 added subsequently as a preservative.

The vaccines prepared from fluid medium consisted of whole culture, diluted with saline to 10 billion organisms per ml. These antigens were killed and preserved by merthiolate 1:10,000; or killed by heating at 56° C. for one hour and then preserved with merthiolate 1:10,000.

The procedure for the mouse protection test has been described in other reports.[2, 3] Three groups of 14 mice each were given graded doses of vaccine in one intraperitoneal injection. After a rest period of 14 days a challenge dose of 100,000 organisms was given intracerebrally. Based on the numbers of mice surviving in the three groups 14 days after challenge, the ED_{50} was calculated. The method used was that described by Litchfield and Fertig[4] in which the standard error of the ED_{50} and of the slope of the dosage mortality curve are calculated as a basis for judging validity of the data.

Results with Vaccines Prepared from Culture Growth on Bordet-Gengou Medium, Stored as Fluid Suspension—One lot of H. pertussis vaccine, number 213, was prepared in March, 1944, divided into four portions, and each killed by a different agent. The results of protection tests on the merthiolate, phenol, formalin, and heat-killed portions of this vaccine after different periods of storage in the cold are shown in Table 1. The end points are expressed in millions of organisms.

It will be seen that the first test recorded was two years and five months after the preparation of the vaccine. Earlier tests were not done by the intracerebral method because it had not yet been standardized. Two years and five months after the vaccine preparation date, there was little difference in the end points of the merthiolate, phenol, and heat-killed portions; the ED_{50} value of the formalin-killed portion was slightly higher. In subsequent tests a recently prepared vaccine was included,

Table 2—Pertussis Vaccine Lot 213: Mouse Protective Properties of Merthiolate, Phenol, Formalin, and Heat-Killed Portions After Storage at Room Temperature

Storage at Room Temperature After 6½ Years in the Cold	Vaccine Lot 213 from Bordet-Gengou Medium								M213 in the Cold (Reference)
	Merthiolate		Phenol		Formalin		Heat		
	ED/50	Ratio	ED/50	Ratio	ED/50	Ratio	ED/50	Ratio	
0	500	1.0	390	0.8	NG	...	400	0.8	500
7½ months	830	3.3	790	3.2	720	2.9	340	1.4	250
12 months	450	3.2	480	3.4	710	5.1	220	1.6	140
15 months	790	1.1	1,000	1.4	NG	...	980	1.4	710
21 months	1,300	4.1	590	1.8	680	2.1	790	2.5	320

and the ratios of end points for stored vaccines to end points of recent vaccines are recorded. These ratios show a gradual and fairly consistent increase with the increased storage period.

The results suggest that the vaccines stored in the cold retained their mouse protective properties at a fairly high level through at least seven years. In relation to the data on the different killing agents, the heat-killed vaccine gave as good protection to mice, and remained potent as long as did the other antigens; in fact, some advantage for the heat-killed antigen was suggested by the results. It may be noted here that although a study of changes in physical properties of stored vaccines was not a part of the present investigation, it was observed that with prolonged storage, the phenol and formalin-killed antigens became dark in color and difficult to resuspend, while the portions killed by merthiolate and by heat showed little change in appearance.

For purposes of study, an attempt was made to accelerate deterioration of the antigens and thereby exaggerate any differences that might occur among antigens under different conditions. From the vaccines which had been stored in the cold for six and one-half years, portions were removed and stored, respectively, at room temperature and at 35° C. The results of mouse protection tests after varying periods at room temperature are given in Table 2.

The ED_{50} values of the vaccines at room temperature are compared with the merthiolate-killed Lot 213, stored continuously in the cold. The results indicate more rapid loss of protective properties at the higher temperature of storage. In the test made after 21 months at room temperature a recently prepared reference antigen was also included. ED_{50} of this antigen was 100 (not included in the table) and the ratios of the four test vaccines to it were 13, 5.9, 6.8, and 7.9, respectively, giving further evidence of the loss in potency after storage at room temperature.

The ED_{50} values of vaccine stored at an incubator temperature of 35° C. subsequent to six and one-half years in the cold are recorded in Table 3.

The results indicate that the vaccine deteriorated during the first six months at incubator temperature, and after one year and three months there was practically no protection. It should be noted that the ratios relate the end points of

Table 3—Pertussis Vaccine Lot 213: Mouse Protective Properties of Merthiolate, Phenol, Formalin, and Heat-Killed Portions After Storage in the Incubator at 35° C

Storage at 35° C After 6½ Years in the Cold	Vaccine Lot 213 from Bordet-Gengou Medium								M213 in the Cold (Reference)
	Merthiolate		Phenol		Formalin		Heat		
	ED/50	Ratio	ED/50	Ratio	ED/50	Ratio	ED/50	Ratio	
0	500	1.0	390	0.8	NG	...	400	0.8	500
3 months	NG	...	1,700	5.1	620	1.9	500	1.5	330
6 months	1,300	4.1	1,200	3.8	2,300	7.2	620	1.9	320
9 months	220	1.3	680	4.0	500	2.9	NG	...	170
12 months	390	2.8	980	7.0	NG	...	1,000	7.1	140
15 months	2,000	8.3	No protection	...	No protection	...	1,000	4.2	240
21 months	740	2.3	890	2.8	830	2.6	1,000	3.1	320
23 months	NG	...	3,200	8.6	NG	...	1,450	3.9	370
2 years	3,000	6.7	1,950	4.3	NG	...	NG	...	450

the vaccine stored at 35° C. to the end points of the merthiolate-killed portion which had been stored in the cold, M213 (last column in the table). Had the results with the incubator-stored vaccine been compared with a recently prepared lot, even higher ratios could have been expected. It may be noted that the ED_{50} values of the incubator-stored portions varied more from test to test than did those for the vaccines stored in the cold. Comparing the killing agents, when all results are considered, none stands out as definitely superior. In line with a previous notation, however, there is a suggestion, particularly in the tests at three and six months, that the heat-killed vaccine was slightly better than the others.

A weakness of these early experiments was the lack of tests immediately after Lot 213 was harvested; at that time, as indicated earlier, a potency test had not been standardized. It was possible, therefore, that an unobserved loss in antigenicity had occurred immediately after preparation of the vaccine. To test this possibility another lot (No. SA8) was prepared on Bordet-Gengou medium, and potency tests were started four days after harvest and repeated at frequent intervals after storage at 5 to 10° C. The results during a period of five and one-half months are given in Table 4.

While the ED_{50} values of the two vaccines give some indication that the heat-killed lot may have been slightly more potent, there is no indication of deterioration in antigenicity of either vaccine during the five and one-half months of observation.

Results with Vaccine Prepared in a Fluid Medium and Stored as Fluid Suspension—A heat-killed, merthiolate preserved vaccine prepared from culture 10-536 grown in Cohen-Wheeler fluid medium was tested at intervals after

Table 4—Pertussis Vaccine Lot SA8: Mouse Protective Properties at Intervals Beginning at Four Days After Storage in the Cold (5°–10°)

Storage Period	Vaccine Lot SA8 from Bordet-Gengou Medium				
	Merthiolate-Killed		Heat-Killed Merthiolate-Preserved *		Reference
	ED/50	Ratio	ED/50	Ratio	RA8
4 days	760	2.3	280	0.8	330
19 days	250	1.5	450	2.6	170
34 days	510	1.3	300	0.8	400
54 days	600	3.3	220	1.2	180
69 days	400	2.0	NG	...	200
5½ months	150	0.4	140	0.4	350

* Merthiolate added subsequent to heating

storage in the cold (5°–10°) and in the incubator at 35° C. The results are given in Table 5.

After storage in the cold the vaccine showed no loss of potency when last tested, at 15 months. After storage in the incubator, however, marked deterioration was indicated by tests at four months.

Comparative Results with a Vaccine Stored as a Fluid Suspension, and the Same Vaccine Stored in the Lyophilized State—A lot of vaccine prepared in March, 1945, killed and preserved with merthiolate, and adjusted to 10 billion per ml, was divided into two parts: one was stored as a fluid suspension in the cold in the usual manner; the other was dispensed in ampules, frozen and dried by the lyophile method and stored at room temperature. Table 6 shows the results of mouse tests on these two anti-

Table 5—Pertussis Vaccine Lot CW-SA9, Prepared in Fluid Medium: Mouse Protective Properties After Different Storage Periods

Storage Period	Heat-Killed Merthiolate-Preserved *				Reference
	Cold Room		Incubator		
	ED/50	Ratio	ED/50	Ratio	RA8
9 days	130	0.7	180	0.9	190
15 days	210	0.6	210	0.6	340
43 days	NG	...	710	2.2	320
50 days	250	1.6	250	1.6	160
4 months	180	0.6	2,000	6.3	320
9 months	110	0.4	NG	...	270
15 months	240	0.6	No protection		380

* Merthiolate added subsequent to heating

Table 6—Pertussis Vaccine: Mouse Protective Properties After Storage in Fluid and Dried State

Storage Period	Fluid ED/50	Fluid Ratio	Dried ED/50	Dried Ratio	Reference ED/50 (Recently Prepared)
13 months	180	1.1	600	3.8	160
14 months	210	0.7	170	0.6	300
2 years	360	2.8	NG	...	130
3 years	220	1.7	210	1.6	130
4 years	320	1.2	330	1.2	270
5 years 8 months	400	0.6	300	0.5	620
7 years	790	...	650	...	NG (roughly 580)
7 years 6 months	400	0.8	630	1.2	510
8 years	400	1.2	210	0.6	340
8 years 10 months	360	0.9	220	0.5	420
9 years 11 months	400	1.0	330	0.8	400

gens after periods of storage up to 10 years.

Whether the ED_{50} values of the fluid and dried antigens are compared with each other or with those obtained with recently prepared reference vaccines, there is no evidence of deterioration up to eight years and nine months after preparation. The averages of the ED_{50} values for fluid, dried and reference antigens are 370, 365, and 350, respectively.

Discussion

Killing Agents—Although the experiments included comparative testing of vaccines killed with several agents, they give no clear-cut answer that one agent assures a more potent product than another. It is noteworthy that the data indicate no difference either in mouse protective properties when first prepared, or in stability between vaccines killed by merthiolate and those killed by heat. Perhaps the use of heat as a killing agent in the preparation of pertussis vaccines needs reconsideration, since there are certain obvious advantages, particularly in relation to toxicity. Further basis may be provided by current studies in other laboratories on the nature of the antigen responsible for protection. In the choice of a killing agent there are, of course, practical considerations such as its effect on the physical state, including ease of resuspension and color. Differences in these respects were noted earlier for the several agents studied.

Vaccines from Growth in Fluid Media—In relation to vaccines prepared in fluid media, even when mouse protection tests have indicated good antigenicity, there has been some concern as to whether they would maintain their antigenicity during storage. The fact that the vaccine tested maintained its mouse-protective properties for 15 months in the cold gives reason for expecting it to be stable much longer. In general, if a vaccine has given good protection

of mice in early tests it has maintained its protective properties for a matter of years under conditions of cold storage.

Stability of a Vaccine Dried from the Frozen State—The data indicate stability of a dried vaccine stored at room temperature for many years. While it is true that vaccine in fluid suspension showed remarkable stability under controlled conditions of storage, there are obvious advantages in a dried product for reference use on a large scale—as an international reference antigen, for example. Wide use of dried reference antigens, now available from the National Institutes of Health, will add much information as experience accumulates.

Summary

The results of comparative mouse protection tests over a period of years have been reported for pertussis vaccines prepared from cultures grown and killed by various methods and stored under different conditions. Stored in the cold, some of the vaccines retained mouse protective properties for as long as from eight to 10 years. The results suggest that heat at 56° C for an hour might well be reconsidered as a killing method. A vaccine dried from the frozen state showed no evidence of deterioration after 10 years; the suitability of such a preparation for a reference vaccine is discussed.

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Surplus Property for Health Uses

A surplus property bill (HR 3322) has been passed by Congress and signed by the President. It makes large quantities of government surplus available for donation to health departments, hospitals, and professional schools and with more liberal terms of transfer than heretofore.

The Department of Health, Education and Welfare through its Division of Surplus Property Utilization has published a "how-to-acquire-surplus" guidebook for those who desire to acquire surplus property for health or educational use. Its title—Acquiring Surplus Property for Health or Educational Use. Government Printing Office, Washington 25, D. C.; 15 cents.