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In countries where vaccination against swine erysipelas has been practiced during the last half century, there is a consensus among veterinarians that the best results are obtained when the live culture-vaccine is not only fresh and highly virulent but is endowed also with a good capacity for growth on artificial culture media. Apparently avirulent culture-vaccines are apt to engender only a transitory immunity or fail altogether.

Profiting from the experience acquired elsewhere, culture-vaccines used in the control of swine erysipelas in Nebraska were periodically challenged for their validity by the inoculation of laboratory animals and by bacteriologic examinations. In the latter, a number of the vaccines examined failed to disclose dependable qualities. Of the 250 culture-vaccine samples tested during the period 1942–1945, only 64 per cent proved to be acceptable for vaccination purposes.* Some improvement became apparent in 1945 when, of the 70 samples tested, 72 per cent proved to be dependable.

The results of tests indicated further that, whereas a number of producers more or less constantly succeeded in the preparation of acceptable materials, certain others failed to do so.

The problem presented by undesirable culture-vaccines was deemed to be of such importance to the swine industry that this Station undertook a series of experiments, to determine what influences might be accountable for the production of inferior vaccination materials.

Modes of Procedure

Culture-vaccines were prepared in accordance with either prescribed Standard † methods or with such variation as is set forth in the more detailed descriptions of the various groups of experiments which are to follow.

Some of these variations were based on reports by producers who attributed improvements of their culture-vaccines to certain specified influences, or who were anxious to know whether or not such factors as the brand of peptone used in the preparation of culture-broth may have been responsible for unfavorable results. Other factors were also mentioned by them, such as the types of glassware used for storage,

* 58th Annual Report of the Nebraska Agricultural Experiment Station, p. 86–1944. 1945.

† Standard cultures are prepared as follows: 500 grams of minced fresh beef heart mixed in one liter distilled water is held for 24 hours in a refrigerator, sterilized, meat removed by straining. Ten grams peptone and 8.5 grams NaCl added, filtered through paper and pH adjusted to 8.0.

the length of time between pigeon passage and the culture inoculations, or the influence of agitation of the vaccines while in transit, as well as the tainting of basic beef hearts used in their preparation.

In the preparation of the culture-vaccines for experimental purposes, only strains No. 87184 and 87193 of *E. rhusiopathiae* were used.

The growth capacity was determined as follows. Just prior to the inoculation of the pigeons, a loopful of culture vaccine was inoculated on two beef-heart agar slants; the condensation water was washed over the slant surface, incubated at 37.5°C. for 48 hours, and then examined.

These strains, inoculated in beef-heart broth, were incubated for 48 hours or otherwise in accordance with the plan of the separate experiments.

Each culture strain was incubated in separate containers and, after the completion of incubation, the broth cultures were mixed in equal parts, bottled in 30 cc. vials, and stored in the cooler or otherwise to conform with the plan of the different experiments. A total of 13 such vials of culture-vaccine was used in each experiment.

Immediately after the bottling of the cultures, sub-cultures were started in order to establish growth capacity, the hydrogen-ion concentration was determined, and four pigeons were inoculated, each with 0.25 cc. of the culture. From then on this procedure was repeated at weekly intervals with the contents of a fresh vial, destroying the remnants after the initiation of each test.

The cause of death of the pigeons which succumbed in the experiments was regularly challenged by examination of their blood or by culture methods.

In order that the results obtained in the various experiments could be adequately compared, each pigeon which died of *E. rhusiopathiae* infection was credited with 2.5 points, thus if all the four pigeons died in a single experiment the group was credited with ten points.

The final results were recorded in tabular form as the average of points and other data obtained in the course of three periods of four weeks each.

Analysis of the Average Validity, Hydrogen-ion Concentration, Growth Capacity, and Approximate Incubation Periods, by Periods of Four Weeks Each

TABLE I.—REGULAR CULTURE-VACCINES.

Standard Cultures (10) Nos. C43876—C47248—C53902—C55906—C57476—C57477—C57478—C57489—C59428—C59966 prepared between 1–15–43 and 2–6–45.

Ratings of:	1st period	2nd period	3rd period
Validity	93.75%	86.25%	78.75%
Hydrogen-ion concentration	8.2	8.0	8.0
Growth capacity	3+	2.8+	2.7+
Approximate length of incubation (in days)	3.9	4.1	4.3

TABLE II.—THE POSSIBLE INFLUENCE OF DEXTROSE.

Irregular Cultures (7) Nos. C43877—C47249—C47534—C47582—C49840—C50581—C50875 prepared between 1–15–43 and 10–30–43. Grown on broth containing 0.5 per cent glucose.

Ratings of:	1st period	2nd period	3rd period
Validity	80.35%	68.75%	56.25%
Hydrogen-ion concentration	6.2	6.2	6.1
Growth capacity	2.7+	2.3+	2.0+
Approximate length of incubation (in days)	4.5	4.0	4.0

TABLE III.—THE POSSIBLE INFLUENCE OF YEAST TREATMENT.

Irregular Cultures (7) Nos. C43878—C47250—C47535—C47583—C49841—C50582—C50876 prepared between 1–15–43 and 10–30–43. Grown on broth treated with yeast, in order to eliminate all fermentable sugars.

Ratings of:	1st period	2nd period	3rd period
Validity	91.08%	70.52%	74.10%
Hydrogen-ion concentration	7.7	7.7	7.6
Growth capacity	2.5+	2.2+	2.2+
Approximate length of incubation (in days)	4.5	5.1	4.8

TABLE IV.—THE POSSIBLE INFLUENCE OF LIVER BROTH.

Irregular Cultures (2) Nos. C53903 and C53904 prepared on 3–21–44. These cultures were grown in liver broth as follows: No. C53903—broth composed of equal parts of plain beef heart broth and equal parts of liver broth. No. C53904 was grown in liver broth exclusively.

Culture Nos.	Ratings of:	1st period	2nd period	3rd period
C53903	Validity	75.0%	6.25%	None
	Hydrogen-ion concentration	5.4	5.3	5.4
	Growth capacity	1.7+	None	None
	Approximate length of incubation (in days)	4.5	5.0	Not determined
C53904	Validity	31.25%	None	None
	Hydrogen-ion concentration	5.3	5.4	5.4
	Growth capacity	1.2+	None	None
	Approximate length of incubation (in days)	4.8	Not determined	

TABLE V.—THE POSSIBLE INFLUENCE OF PRODUCER'S BROTH.

Irregular Cultures (2) Nos. C47533 and V47581 prepared on 6–4–43. These cultures were grown in broth supplied by producers.

No. C47533 was grown on regular broth supplied by a producer whose culture-vaccines frequently proved to be sub-standard (A).

No. C47581 was grown on regular broth by a producer whose culture-vaccines were nearly always of excellent quality (B).

Culture Nos.	Ratings of:	1st period	2nd period	3rd period
C47533	Validity	100%	62.50%	31.25%
	Hydrogen-ion concentration	6.9	6.9	6.8
	Growth capacity	2.7+	3+	2+
	Approximate length of incubation (in days)	4.2	4.9	5.2
C47581	Validity	93.75%	93.75%	87.50%
	Hydrogen-ion concentration	6.9	6.9	6.9
	Growth capacity	2.7+	3.0+	3.0+
	Approximate length of incubation (in days)	3.9	4.7	4.5

TABLE VI.—THE POSSIBLE INFLUENCE OF GLASSWARE TYPES.

Irregular Cultures (3) Nos. C54040—C54041—C54042 prepared on 3-24-44 by standard methods to determine a possible influence by the type of glassware in which they were stored.

Cultures No. C54040 were stored in No. 1845 glass vials.
Cultures No. C54041 were stored in borosilicate glass vials.
Cultures No. C54042 were stored in common amber glass vials.

Culture Nos.	Ratings of:	1st period	2nd period	3rd period
C54040	Validity	100%	100%	87.5%
	Hydrogen-ion concentration	8.1	7.9	8.0
	Growth-capacity	2.5+	3+	2+
	Approximate length of incubation (in days)	4.0	4.1	4.4
C54041	Validity	100%	93.75%	87.50%
	Hydrogen-ion concentration	8.1	8.0	8.0
	Growth capacity	3+	3+	2+
	Approximate length of incubation (in days)	3.7	4.0	4.0
C54042	Validity	81.25%	100%	93.75%
	Hydrogen-ion concentration	8.1	8.0	8.0
	Growth capacity	3+	2.5+	2+
	Approximate length of incubation (in days)	4.0	4.5	4.2

TABLE VII.—THE POSSIBLE INFLUENCE OF PEPTONES.

Irregular Cultures (3) Nos. C55906—C55907—C55908 prepared on 6–25–44 in the following manner:

No. C55906—cultures grown in broth containing peptone A.

No. C55907—cultures grown in broth containing peptone B.

No. C55908—cultures grown in broth without peptone.

Culture Nos.	Ratings of:	1st period	2nd period	3rd period
C55906	Validity	100%	75%	68.75%
	Hydrogen-ion concentration	8.4	8.3	8.3
	Growth capacity	3+	2.7+	2+
	Approximate length of incubation (in days)	3.9	4.9	4.3
C55907	Validity	100%	93.75%	75%
	Hydrogen-ion concentration	8.1	8.1	8.2
	Growth capacity	3+	2.7+	2.0+
	Approximate length of incubation (in days)	3.8	3.8	4.5
C55908	Validity	87.5%	87.5%	43.75%
	Hydrogen-ion concentration	6.9	6.9	6.9
	Growth capacity	3+	2.7+	1.7+
	Approximate length of incubation (in days)	4.6	3.9	5.4

TABLE VIII.—THE POSSIBLE INFLUENCE OF PIGEON PASSAGES.

Irregular Cultures (3) Nos. C57476—C57477—C57478 prepared on 9-28-44 in accordance with the following plan:

No. C57476—cultures started seven days after pigeon passage.

No. C57477—cultures started 60 days after pigeon passage.

No. C57478—cultures started 120 days after pigeon passage.

Culture Nos.	Ratings of:	1st period	2nd period	3rd period
C57476	Validity	100%	100%	100%
	Hydrogen-ion concentration	8.1	8.0	8.0
	Growth capacity	3+	3+	3+
	Approximate length of incubation (in days)	3.6	3.9	3.7
C57477	Validity	100%	100%	87.50%
	Hydrogen-ion concentration	8.1	8.0	7.9
	Growth capacity	3+	3+	3+
	Approximate length of incubation (in days)	3.9	4.2	4.0
C57478	Validity	93.75%	93.75%	100%
	Hydrogen-ion concentration	8.1	7.9	7.9
	Growth capacity	3+	3+	2.7+
	Approximate length of incubation (in days)	3.4	3.8	4.0

TABLE IX.—THE POSSIBLE INFLUENCE OF THE ADDITION OF GLUCOSE AND YEAST TREATMENT.

These cultures were treated as follows:
Culture No. C50580 prepared in accordance with prescribed standards.
Culture No. C50581 prepared from broth containing 0.5 per cent glucose.
Culture No. C50582 prepared from yeast-treated broth.
(All these cultures were incubated at room temperature and stored in cooler.)

Culture Nos.	Ratings of:	1st period	2nd period	3rd period
C50580	Validity	68.75%	81.25%	93.75%
	Hydrogen-ion concentration	8.1	8.1	8.2
	Growth capacity	3+	2.2+	2.7+
	Approximate length of incubation (in days)	4.0	4.7	4.6
C50581	Validity	87.5%	100%	100%
	Hydrogen-ion concentration	6.9	6.9	6.9
	Growth capacity	2.7+	2.5+	2.7+
	Approximate length of incubation (in days)	4.1	4.5	3.6
C50582	Validity	100%	93.75%	100%
	Hydrogen-ion concentration	7.8	7.8	7.8
	Growth capacity	2.2+	2.0+	2.7+
	Approximate length of incubation (in days)	3.7	3.8	4.3

TABLE X.—THE POSSIBLE INFLUENCE OF THE ADDITION OF GLUCOSE YEAST TREATMENT AND DIFFERENCES IN INCUBATION AND STORAGE.

These cultures were treated as follows:
Culture No. C50874 prepared in accordance with standard methods.
Culture No. C50875 prepared from broth containing 0.5 per cent glucose.
Culture No. C50876 prepared from yeast-treated broth.
(These cultures were incubated for 48 hours and then stored at room temperature.)

Culture Nos.	Ratings of:	1st period	2nd period	3rd period
C50874	Validity	100%	87.5%	93.75%
	Hydrogen-ion concentration	7.9	7.9	7.8
	Growth capacity	2.7+	2+	2.7+
	Approximate length of incubation (in days)	4.7	4.4	4.0
C50875	Validity	81.25%	62.5%	81.25%
	Hydrogen-ion concentration	5.5	5.5	5.5
	Growth capacity	2.5+	1.5+	1.2+
	Approximate length of incubation (in days)	5.4	4.2	4.5
C50876	Validity	93.75%	100%	37.5%
	Hydrogen-ion concentration	7.9	7.8	7.8
	Growth capacity	2+	1.7+	2.7+
	Approximate length of incubation (in days)	4.6	4.1	4.4

TABLE XI.—THE POSSIBLE INFLUENCE OF INCUBATION AND STORAGE TEMPERATURES.

One standard culture and two irregular ones, Nos. C57489—C57490—C57491, prepared on 9–28–44, treated in the following manner:

No. C57489 prepared according to standard methods for comparison.

No. C57490—culture incubated at room temperature and stored in cooler.

No. C57491—culture incubated and stored at room temperature.

Culture Nos.	Ratings of:	1st period	2nd period	3rd period
C57489	Validity	100%	100%	100%
	Hydrogen-ion concentration	8.0	8.0	7.9
	Growth capacity	3+	3+	2.7+
	Approximate length of incubation (in days)	3.8	4.0	4.0
C57490	Validity	100%	100%	100%
	Hydrogen-ion concentration	8.0	7.8	7.6
	Growth capacity	3+	3+	3+
	Approximate length of incubation (in days)	4.0	4.0	3.6
C57491	Validity	100%	87.5%	100%
	Hydrogen-ion concentration	7.8	7.6	7.6
	Growth capacity	3+	3+	2.5+
	Approximate length of incubation (in days)	4.1	4.0	4.0

TABLE XII.—THE POSSIBLE INFLUENCE OF AGITATION.

One standard culture and two irregular ones, Nos. C59428—C59429—C59430, prepared on 1–12–45, were subjected to the following treatment.

No. C59428 prepared according to standard methods and not subjected to shaking.

No. C59429 prepared according to standard methods and subjected to shaking for six hours on two successive days immediately preceding pigeon inoculation.

No. C59430 prepared according to standard methods and subjected to shaking for six hours on three successive days immediately preceding pigeon inoculations.

Culture Nos.	Ratings of:	1st period	2nd period	3rd period
C59428	Validity	81.25%	93.75%	81.25%
	Hydrogen-ion concentration	7.9	7.3	7.3
	Growth capacity	3+	3+	3+
	Approximate length of incubation (in days)	4.8	3.4	4.4
C59429	Validity	100%	93.75%	93.75%
	Hydrogen-ion concentration	7.7	7.4	7.6
	Growth capacity	3+	3+	2.7+
	Approximate length of incubation (in days)	3.6	4.0	4.4
C59430	Validity	100%	100%	87.50%
	Hydrogen-ion concentration	7.8	7.5	7.7
	Growth capacity	3+	3+	2.7+
	Approximate length of incubation (in days)	4.4	3.4	3.6

TABLE XIII.—THE POSSIBLE INFLUENCE OF FAT CONTENTS.

Irregular Cultures (3) Nos. C59758—C59759—C59760 prepared on 1–27–45. The cultures were prepared as follows:

No. C59758—the beef heart used in the culture broth had all fat carefully removed.

No. C59759—the culture broth was composed of equal parts of culture media, Nos. C59758 and C59760.

No. C59760—the beef heart used in the culture broth had none of the fat removed.

Culture Nos.	Ratings of:	1st period	2nd period	3rd period
C59758	Validity	100%	87.5%	100%
	Hydrogen-ion concentration	8.0	8.0	8.0
	Growth capacity	3+	3+	3+
	Approximate length of incubation (in days)	3.0	3.9	3.8
C59759	Validity	87.5%	93.75%	100%
	Hydrogen-ion concentration	7.8	7.8	7.8
	Growth capacity	3+	3+	3+
	Approximate length of incubation (in days)	3.4	3.6	3.1
C59760	Validity	93.75%	93.75%	93.75%
	Hydrogen-ion concentration	7.6	7.6	7.5
	Growth capacity	3+	3+	3+
	Approximate length of incubation (in days)	3.9	3.5	3.6

TABLE XIV.—THE POSSIBLE INFLUENCE OF BEEF HEART TAINTING.

Irregular Cultures (3) Nos. C59966—C59967—C59968 prepared on 2-6-45 in the following manner:

No. C59966—medium made with beef heart secured immediately after slaughter.

No. C59967—medium made with beef heart kept at room temperature during 24 hours.

No. C59968—medium made with beef heart kept at room temperature during 48 hours.

Culture Nos.	Ratings of:	1st period	2nd period	3rd period
C59966	Validity	100%	87.50%	93.75%
	Hydrogen-ion concentration	8.5	8.4	8.3
	Growth capacity	3+	3+	2.7+
	Approximate length of incubation (in days)	3.4	4.5	4.3
C59967	Validity	93.75%	75.0%	87.50%
	Hydrogen-ion concentration	8.7	8.7	8.6
	Growth capacity	3+	3+	2.7+
	Approximate length of incubation (in days)	4.1	4.8	4.2
C59968	Validity	93.75%	87.50%	87.50%
	Hydrogen-ion concentration	8.8	8.8	8.8
	Growth capacity	3+	3+	2.7+
	Approximate length of incubation (in days)	3.8	4.3	4.4

Notes and Comments

DETAILED FINDINGS are recorded in Tables I–XIV. Validity ratings are also exhibited by Figures 1–34. Prior to the inoculation of the culture broth, hydrogen-ion concentration was adjusted to pH 8.0 for all the series with the exception of the two broths which were supplied by producers.

In the actual experiments, the hydrogen-ion concentration showed some variations but, with the exception of cultures containing glucose and the ones growing in liver broth, the pH did not appear to have an influence on culture validity. This influence was also apparent in the decline or even loss of growth capacity in the series concerned, With these exceptions, growth capacity remained within normal limits. In some of the series a progressive and sometimes an irregular decline became apparent without this becoming objectionable. Pigeon influence and aging may have been factors in these phenomena.

Owing to the fact that no observations could be made between 5 p.m. and 8 a.m., the incubation period after pigeon inoculation could be only roughly approximated, because birds dying before 12 a.m. were recorded as belonging to the following day. Hence the recorded periods in some of the cases were longer than they should have been. Throughout the entire series, these incubation periods remained within acceptable limits with an over-all average of slightly more than four days.

Standard culture vaccines. Interspersed in the general series were ten culture-vaccines prepared in accordance with standard methods in order to establish a base for comparison. Culture validity was determined as averages attained in each of the three periods of four weeks each. The average validity of the ten culture-vaccines proved to be acceptable throughout. However, one of the components of this group had become sub-valid in the second and third periods, and two additional cultures had become non-acceptable in the third period. Slight, progressive declines in the hydrogen-ion concentration became apparent as the stored cultures aged, and more or less increase in the approximated incubation periods shown by the pigeons may be attributed to the same cause (See Table I and Figure 1).

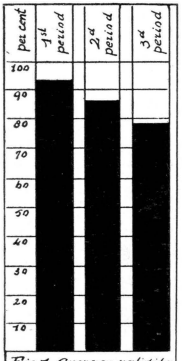

Fig. 1. Average validity of ten Standard culture vaccines.

The influence of glucose. A group of seven cultures was grown in broth containing 0.5 per cent glucose. The average validity of this group was acceptable only in the first period and failed to attain a desirable rating in the other two. The hydrogen-ion concentration dropped from the pre-inoculation titer pH 8.0 to a general average of pH 6.2. Growth capacity showed but a moderate decline without becoming sub-standard. Conceivably the low validity of the culture-

vaccines may be attributed to the marked acidity derived from the presence of glucose (See Table II and Figure 2).

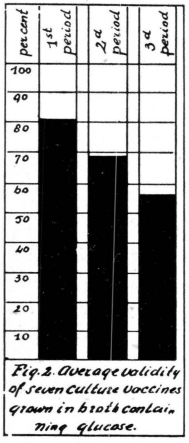

Fig. 2. Average validity of seven culture vaccines grown in broth containing glucose.

The influence of yeast treatment. A group of seven culture-vaccines was grown in broth which had previously been treated with yeast, in order to eliminate fermentable sugars which may have been present in the beef hearts used in preparation of the culture-vaccines. The average validity of this group was acceptable in the first period and slightly sub-valid in the other two periods. There was a moderate, progressive reduction of the hydrogen-ion concentration. It seemed possible that certain by-products of fermentation may have been accountable for the minor validity displayed in the second and third periods (See Table III and Figure 3).

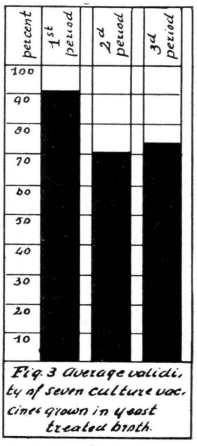

Fig. 3 Average validity of seven culture vaccines grown in yeast treated broth.

The influence of liver broth. It had been observed that *E. rhusiopathiae* developed luxuriously in liver broth, and it was deemed possible that this type of medium might enhance the validity of cultures grown in it. That the contrary was the case was demonstrated by the following experiments.

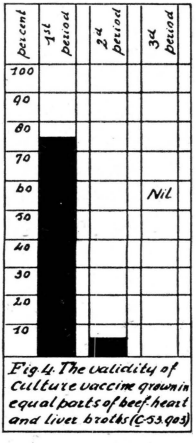

Fig 4. The validity of Culture vaccine grown in equal parts of beef heart and liver broths (C-53903)

Culture No. C53903, grown on broth composed of equal parts of beef heart and liver broth. The validity of this product was found to be acceptable just in the first period. It showed an inconsequential validity in the second period and none at all in the third. The hydrogen-ion concentration was changed from the preculture inoculation of pH 8.0 to a general average of pH 5.4. The growth capacity in the first period was far below standard and nil in the other two (See Table IV and Figure 4).

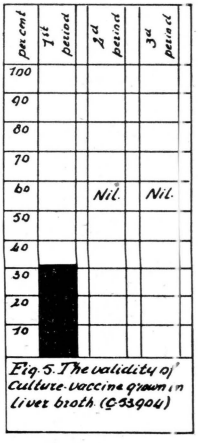

Fig. 5. The validity of Culture-vaccine grown in liver broth (C-53904)

Culture No. C53904, grown in liver broth only. This culture vaccine showed a minor validity in the first period and none in the second and third periods. Its growth capacity was markedly deficient

in the first period and was nil in the other two (See Table IV and Figure 5).

A study of broth culture media from commercial producers. Two producers, A and B, of whom one frequently failed to produce acceptable culture-vaccine and the other constantly prepared valid materials, supplied quantities of their broth media for experimental purposes. The following is an account of the results of these experiments.

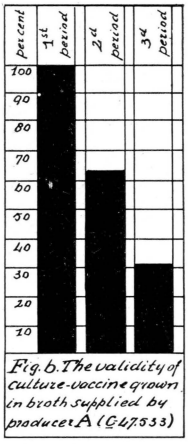

Fig. 6. The validity of culture-vaccine grown in broth supplied by producer A (C47533)

Culture No. C47533. This culture-vaccine showed a maximum validity in the first period but proved to be non-acceptable in the other two periods. The hydrogen-ion concentration was more or less stable at pH 6.9 (See Table V and Figure 6).

Culture No. C47581 presented a rather high validity in all of the three periods. Its hydrogen-ion concentration was identical to the one ascertained in the preceding experiments (See Table V and Figure 7).

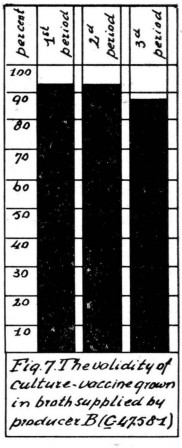

Fig. 7. The validity of culture-vaccine grown in broth supplied by producer B (C47581)

The influence of glass used for storage. Because it was suggested that the glass in which culture-vaccines were being stored may in some manner exercise an influence on bacterial longevity, the following experiments were undertaken:

Culture No. C54040, stored in No. 1845 glass vials.

Culture No. C54041, stored in borosilicate glass vials.

Culture No. C54042, stored in common amber glass vials.

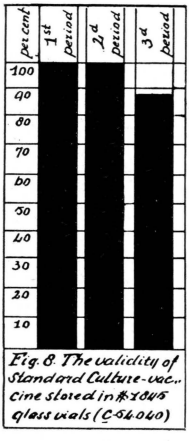

Fig. 8. The validity of standard culture-vaccine stored in #1845 glass vials (C54040)

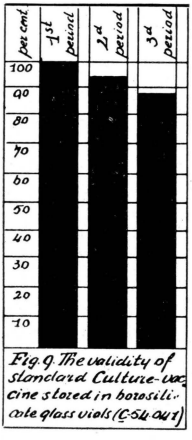

Fig. 9. The validity of standard culture-vaccine stored in borosilicate glass vials (C54041)

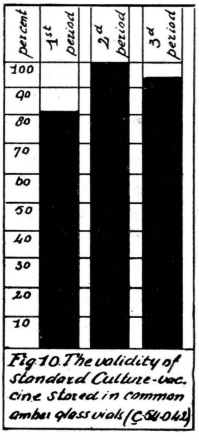

Fig. 10. The validity of standard culture-vaccine stored in common amber glass vials (C54042)

These cultures were prepared by standard methods. All these culture-vaccines displayed a high degree of validity in all of the three periods. Hydrogen-ion concentration remained slightly above pH 8.0 in the entire series. It became apparent that validity of the culture-vaccine was not materially influenced by the type of glass in which it was stored (See Table VI and Figures 8–9–10).

The influence of peptone. Attention being called to the possibility that the nature of the peptone used in the preparation of culture-broth may be responsible for variation in the validity of culture-vaccines, the following experiments were designed to throw light on the subject:

Culture No. C55906, culture broth prepared with peptone A which has been used as a routine procedure throughout all the other experiments. The culture-vaccines thus prepared revealed a maximum validity in the first period, a barely acceptable one in the second, and a sub-standard quality in the third period. The hydrogen-ion concentration average pH 8.3 for the three periods and growth capacity progressively declined without becoming subnormal (See Table VII and Figure 11).

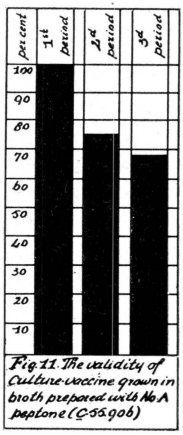

Fig. 11. The validity of culture-vaccine grown in broth prepared with No. A peptone (C55906)

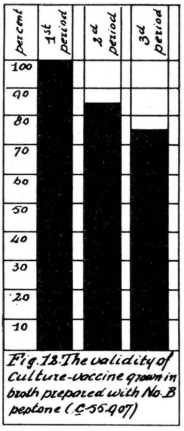

Fig. 12. The validity of culture-vaccine grown in broth prepared with No. B peptone (C55907)

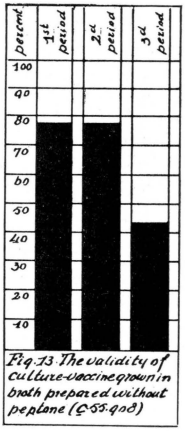

Fig. 13. The validity of culture-vaccine grown in broth prepared without peptone (C55908)

Culture No. C55907, broth prepared with peptone B. This product revealed an acceptable validity in the three periods. The hydrogen-ion concentration showed but a slight fluctuation with an average pH 8.1 for all periods (See Table VII and Figure 12).

Culture No. C55908, prepared without peptone. This culture-vaccine had an acceptable validity in the first and second periods, whereas it had become markedly sub-standard in the third. The hydrogen-ion concentration remained slightly below pH 7.0. Growth capacity was normal in the first two periods but had become sub-standard in the third (See Table VII and Figure 13).

Comment. There was apparently a difference in the qualities of the two peptones in favor of peptone B. The results obtained with

culture-vaccine prepared without peptone point to the value of peptone in the maintenance of bacterial longevity. This suggests that a reduction in the peptone contents of the culture broth may occasionally exercise an unfavorable influence on the validity of culture-vaccine.

The influence of pigeon passage. In this experimental series, an effort was made to determine a possible influence on culture-vaccine validity by the length of time elapsing between pigeon passages of the culture used. The latter were prepared in accordance with standard methods and the following mode of procedure was carried out:

Culture No. C57476, started seven days after pigeon passage.

Culture No. C57477, started 60 days after pigeon passage.

Culture No. C57478, started 120 days after pigeon passage.

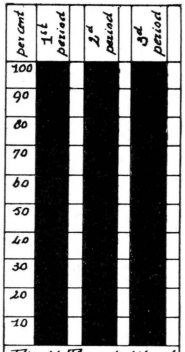

Fig. 14. The validity of culture-vaccine started seven (7) days after pigeon passage (C57476)

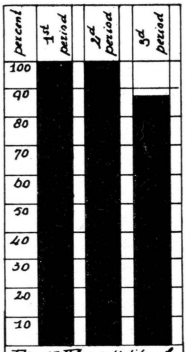

Fig. 15. The validity of culture-vaccine started sixty (60) days after pigeon passage (C57477)

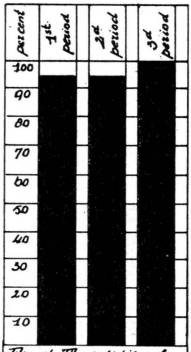

Fig. 16. The validity of culture-vaccine started one hundred and twenty (120) days after pigeon passage (C57478)

The results of these experiments disclosed that in each of the three groups a high validity was attained in the three periods. The hydrogen-ion concentration showed only slight fluctuations around pH 8.0 (See Table VIII and Figures 14, 15, and 16).

Comment. On the whole, there were no significant differences disclosed in the three groups, even if the seven-day passage cultures were somewhat superior to the other two categories.

The influence of room temperature incubation. In the following experiments, an attempt was made to establish the influence of room temperature on culture validity, when culture-vaccines were prepared in accordance with standard methods, when grown in a medium containing 0.5 per cent glucose, or after yeast treatment of the broth prior to inoculation. The following mode of procedure was adhered to:

Culture No. C50580, prepared by standard methods, incubated at room temperature, and stored in cooler. This culture-vaccine lacked validity in the first period, but in the second and third periods it

proved to be acceptable. The hydrogen-ion concentration was slightly above pH 8.0, with a general average of pH 8.1 for all periods (See Table IX and Figure 17).

Culture No. C50581, grown in broth containing 0.5 per cent glucose, incubated at room temperature and stored in cooler. This product was acceptable in all three periods, attaining maximum validity in the second and third periods. The hydrogen-ion concentration remained slightly below pH 7.0 with a general average of pH 6.9 for all periods (See Table IX and Figure 18).

Culture No. C50582, grown in yeast-treated broth, incubated at room temperature, and stored in cooler. This culture-vaccine displayed a high degree of validity, which reached the maximum in the first and third periods. Its hydrogen-ion concentrates varied slightly below pH 8.0, with pH 7.8 as a general average for the three periods (See Table IX and Figure 19).

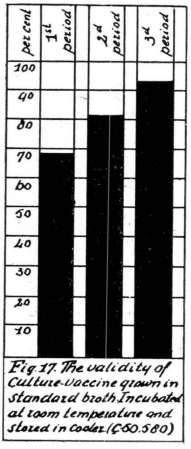

Fig 17. The validity of Culture-Vaccine grown in Standard broth Incubated at room temperature and stored in cooler (C-50.580)

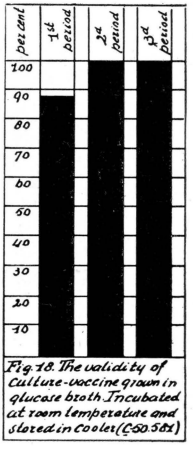

Fig 18. The validity of Culture-Vaccine grown in glucose broth Incubated at room temperature and stored in cooler (C-50.581)

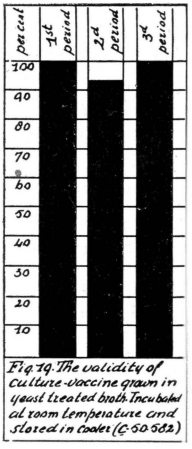

Fig 19. The validity of Culture-Vaccine grown in yeast treated broth Incubated at room temperature and stored in cooler (C-50.582)

Comment. It appears that cultures grown at room temperature and stored in the cooler, despite the influence of glucose and yeast treatment, acquired and maintained a validity.

The following experiments were designed to determine the influence of room temperature storage on the validity of culture-vaccines incubated at 37.5°C. for 48 hours, when they were prepared by standard method or when grown in glucose and yeast-treated broths. The mode of procedure was as follows:

Culture No. C50874, incubated at 37.5°C. and stored at room temperature. This product had an excellent validity with a maximum in the first period. The hydrogen-ion concentration remained slightly below pH 8.0 with a general average of pH 7.9 for all periods (See Table X and Figure 20).

Culture No. C50875, grown in broth containing 0.5 per cent glucose, incubated at 37.5°C., and stored at room temperature. This

culture-vaccine showed a moderate validity in the first and third periods but was sub-standard in the second one. The hydrogen-ion concentration had changed from pre-inoculation pH 8.0 to pH 5.4 as an average for the three periods. Growth capacity proved to be normal in the first period only, and declined sharply to sub-standard ratings in the second and third (See Table X and Figure 21).

Culture No. C50876, grown in yeast-treated broth. This product manifested a high validity in the first and second periods but made a lamentable showing in the third one. The hydrogen-ion concentration remained slightly below pH 8.0 with a general average of pH 7.8 for all periods. Growth capacity was normal in the first and second periods but had fallen below standard requirements in the third one (See Table X and Figure 22).

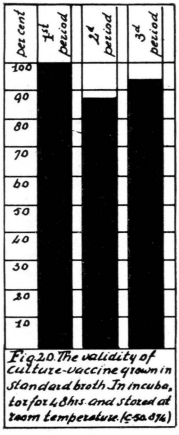

Fig. 20. The validity of culture-vaccine grown in standard broth. In incubator for 48 hrs. and stored at room temperature. (C-50-874)

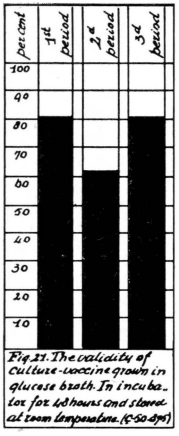

Fig. 21. The validity of culture-vaccine grown in glucose broth. In incubator for 48 hours and stored at room temperature. (C-50-875)

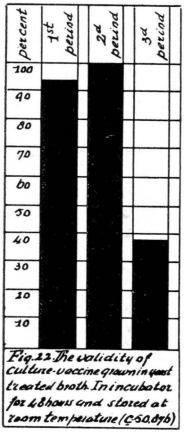

Fig. 22. The validity of culture-vaccine grown in yeast treated broth. In incubator for 48 hours and stored at room temperature (C-50-876)

Comments. It was apparent that room temperature storage did not impair the validity of culture-vaccine prepared by standard methods. On the other hand, the culture-vaccine grown in glucose broth attained but a barely acceptable rating. It seems that the marked acidity of the culture medium during the growth period may have been responsible. However, the relatively poor showing made by the culture grown in yeast-treated broth cannot be readily explained. It is possible that incubation at 37.5°C. may have been accountable for the difference in the results in the preceding groups and the one here considered.

The following is a series of experiments designed to determine the influence on culture-vaccine validity by room temperature incubation and storage in cooler, as well as by room temperature incubation and storage as compared with culture-vaccine prepared in accordance with standard methods.

Culture No. C57489, prepared by standard methods (incubation at 37.5°C.) for comparison. This culture-vaccine displayed a maximum

validity throughout the three periods. The hydrogen-ion concentration was maintained at approximately pH 8.0 with a general average of pH 7.9 for the three periods (See Table XI and Figure 23).

Culture No. C57490, incubated at room temperature and stored in cooler. This product also attained a maximum validity throughout the three periods. Its hydrogen-ion concentration remained close to pH 8.0 with pH 7.8 as a general average for the three periods (See Table XI and Figure 24).

Culture No. C57491, incubated and stored at room temperature. This culture-vaccine also attained a high validity rating throughout the three periods. The hydrogen-ion concentration remained well below pH 8.0 with a general average of pH 7.7 for all the periods (See Table XI and Figure 25).

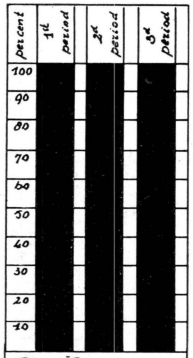

Fig. 23. The validity of culture-vaccine prepared according to standard methods (C-57.489)

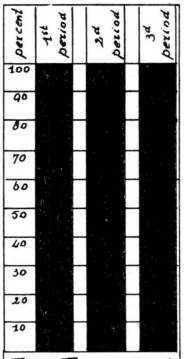

Fig. 24. The validity of culture-vaccine, incubated at room temperature and stored in cooler. (C-57.490)

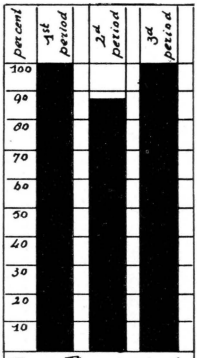

Fig. 25. The validity of culture-vaccine, incubated and stored at room temperature. (C-57.491)

The results of the experiments described above tend to show that culture-vaccines incubated at room temperature and stored in cooler, as well as those incubated and stored at room temperature, attained validity ratings quite comparable with the one presented by culture-vaccine prepared by standard methods.

Comments pertaining to the three preceding series. In the experiments in which room-temperature incubation and storage influences were subjected to analysis, it appeared that these modes of procedure did not prove to be detrimental to culture-vaccine validity. In some of these experiments it developed that, even in cultures grown in broth containing glucose, validity may be quite high. It seemed that the omission of incubation at 37.5°C. may have counteracted the glucose influence on validity (Compare Table II and Figure 2 with Table IX and Figure 18). On the whole, the results indicate that incubation at 37.5°C. in the preparation of culture-vaccines may not be imperative.

The influence of agitation. As it had been suggested that the agitation of culture material, while in transit, may possibly affect culture-vaccine validity, the following experiments were undertaken. All the cultures used in this investigation were prepared in accordance with standard methods.

Culture No. C59428, not shaken.

Culture No. C59429, shaken for six hours for two successive days preceding pigeon inoculation.

Culture No. C59430, shaken for six hours for three successive days preceding pigeon inoculation.

It did not appear that the agitation of the culture-vaccines by rather violent shaking exercised any influence on their validity as in all these series an acceptable quality could be recorded for the three periods. The hydrogen-ion concentration showed only minor variations with a general average of pH 7.6 for the entire group (See Table XII and Figures 26, 27, and 28).

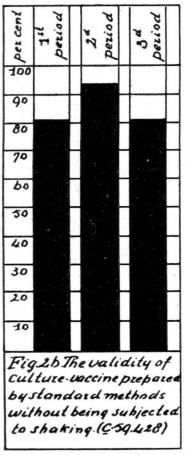

Fig. 26. The validity of culture-vaccine prepared by standard methods without being subjected to shaking. (C59428)

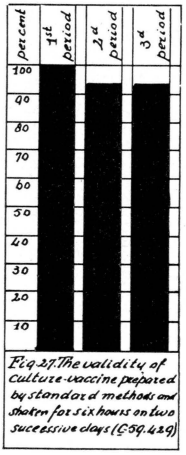

Fig. 27. The validity of culture-vaccine prepared by standard methods and shaken for six hours on two successive days (C59429)

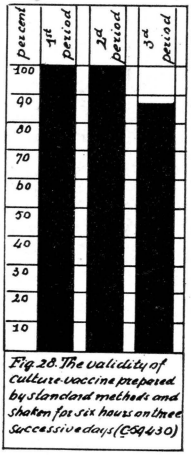

Fig. 28. The validity of culture-vaccine prepared by standard methods and shaken for six hours on three successive days (C59430)

The influence of fat contents. Attention being called to the possibility that the fat content of the beef hearts may in some manner be accountable for undesirable results in the preparation of culture-vaccines, the following attempts were made to ascertain the facts in the matter:

Culture No. C59758, without recourse to chemical fat extraction, the beef heart fat was scrupulously removed prior to mincing.

Culture No. C59759, culture broth composed of equal parts of the broths used in cultures No. C59758 and C59760.

Culture No. C59760, grown in broth prepared with beef heart from which none of the fat had been removed.

These cultures were prepared in accordance with standard methods. Culture-vaccine validity proved to be high in the entire series without

a conspicuous difference *inter se*. The hydrogen-ion concentration in the three groups had a general average of pH 8.0 (See Table XIII and Figures 29, 30, and 31).

Comments. No evidence could be adduced from the obtained results in the above experiments to indicate that the fat content of the beef hearts used had any influence whatsoever on the validity of the culture vaccines prepared in the manner described.

The influence of beef heart decomposition. Because of the fact that decomposition of the beef heart material used in the preparation of culture broth was suspected of being at least one of the causes of validity impairment, efforts were made to determine the extent of such an influence. The following mode of procedure was applied to the problem:

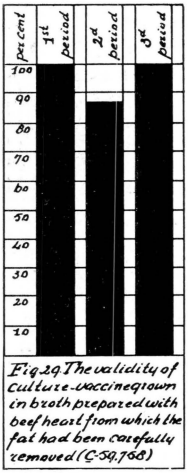

Fig. 29. The validity of culture-vaccine grown in broth prepared with beef heart from which the fat had been carefully removed (C-59,768)

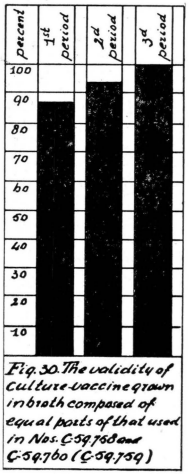

Fig. 30. The validity of culture-vaccine grown in broth composed of equal parts of that used in Nos. C-59,758 and C-59,760 (C-59,759)

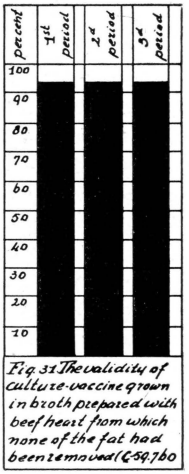

Fig. 31. The validity of culture-vaccine grown in broth prepared with beef heart from which none of the fat had been removed (C-59,760

Culture No. C59966, culture broth prepared in accordance with standard methods with beef hearts secured immediately after slaughter. This culture-vaccine revealed a high degree of validity in all of the three periods. Its hydrogen-ion concentration remained above pH 8.0 with an average of pH 8.4 for the three periods (See Table XIV and Figure 32).

Culture No. C59967, culture broth prepared in accordance with standard methods with beef hearts which were kept at room temperature for 24 hours prior to mincing. At that time there was more or less conspicuous evidence of decomposition. This product also showed a satisfactory validity, although about eight per cent below that prepared with fresh material. The hydrogen-ion concentration was well above pH 8.0, with a general average of pH 8.7 for the three periods (See Table XIV and Figure 33).

Culture No. C59968, culture broth prepared by standard methods with beef hearts which were kept at room temperature for 48 hours prior to mincing. This material was decidedly putrid when used in the preparation of the broth. The validity of this product was acceptable in the three periods, showing a rating slightly over six per cent less than the culture prepared with fresh hearts. It showed a pH 8.8 which was stable for all of the three periods (See Table XIV and Figure 34).

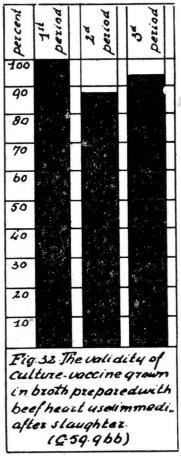

Fig 32. The validity of culture-vaccine grown in broth prepared with beef heart used immediately after slaughter. (C-59.966)

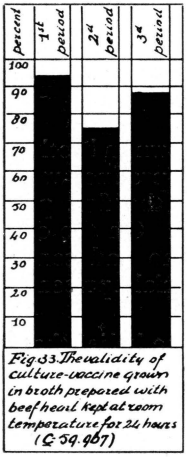

Fig 33. The validity of culture-vaccine grown in broth prepared with beef heart kept at room temperature for 24 hours (C-59.967)

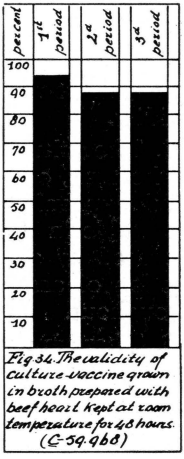

Fig 34. The validity of culture-vaccine grown in broth prepared with beef heart kept at room temperature for 48 hours. (C-59.968)

Comment. Despite the marked decomposition manifested by the beef hearts which had been stored at room temperature, the validity of the culture-vaccines concerned failed to become impaired to a significant degree.

Conclusions

1. The average validity of ten vaccine-cultures prepared in accordance with standard methods was acceptable, even if one of the component culture-vaccines was valid only in the first period and two others had become sub-standard in the third period.

2. The presence of 0.5 per cent glucose in the culture broth exercised a decidedly depressing influence on the average validity of culture-vaccines grown therein.

3. Culture-vaccines prepared with beef heart broth treated with yeast prior to inoculation were of an acceptable validity only in the first period.

4. Culture-vaccines grown in liver broth failed to show acceptable validity.

5. The culture-vaccine grown in broth furnished by a concern which, at the time, often failed to produce valid vaccine material showed a maximum validity only in the first period but proved to be non-acceptable in the other two. On the other hand, the culture-vaccine prepared by a producer who had constantly succeeded in the production of acceptable materials presented a high degree of validity throughout.

6. There is warrant for the belief that the problem connected with sub-valid culture-vaccines can be solved only in the laboratories concerned, rather than by such attempts as described in this publication.

7. Differences were observed in the validity of culture-vaccines grown in broth, each of which was prepared with a different brand of peptone. In one of the two culture-vaccines, the validity was high in the first period, barely acceptable in the second, and of sub-standard quality in the third. In the case of the culture-vaccine grown in broth in which the other specimen of peptone was a part, an acceptable validity was attained in each of the three periods. In a parallel broth prepared without peptone, the culture grown therein was valid in the first and second periods but was markedly sub-valid in the third.

8. The value of peptone in the maintenance of bacterial longevity was demonstrated, and this may suggest that a reduction in the peptone contents of the culture broth may occasionally impair culture-vaccine validity.

9. Room temperature incubation and storage did not prove to be harmful to culture-vaccine validity and apparently incubation at 37.5°C. in the preparation of culture-vaccines may not be a necessity.

10. The nature of glassware used in storage, the length of time after pigeon passage, agitation, the fat content of beef hearts, and a degree of putrification did not appear to influence culture-vaccine validity.