THE DISINFECTANT ACTION OF PHENOL AND OF ALCOHOL IN T.A.B.C. VACCINE*

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The substitution by Felix (1941) of alcohol for phenol in T.A.B.C. vaccine in order to prevent destruction of the Vi antigen represents a considerable advance in the method of prophylactic inoculation against enteric fever. The alcohol, which was employed in a 22.5% or 25% concentration, was intended to fulfil the double purpose of preserving the activity of the Vi antigen and of guarding against infection by virtue of its antiseptic properties. Doubts, however, were soon raised as to its efficiency in this latter respect. Since Felix in his study of the bactericidal action of this substance worked with only one organism, *Staph. aureus*, and since he gave no protocols of any of his experiments, it was felt that the only satisfactory way of allaying these doubts was to carry out a series of observations in which the destructive effect of different strengths of alcohol on different organisms was compared with that of 0.5% phenol.

Technique

Four constituent laboratories of the Emergency Public Health Laboratory Service took part in these trials. Bottles containing 25 ml. of the following T.A.B.C. vaccines were distributed to each laboratory: (a) 0.5% phenol, Batch 18, Oct., 1940, prepared by the Lister Institute; (b) 22.5% alcohol, Batch A1, Sept., 1940, prepared by the Lister Institute; (c) 25% alcohol, Batch AA4, May, 1940, prepared by Dr. Felix.

The experiments were carried out during the summer and autumn of 1941. Observations were made on *Staph. aureus*, *Bact. coli*, *Ps. pyocyanea*, and, in three laboratories, on the spores of *B. subtilis* and *Cl. sporogenes*. With a few exceptions different strains of these organisms were used by different laboratories. The general plan of the experiments was as follows. The three species of non-sporing organisms were grown overnight on agar; the growth was washed off with quarter-strength Ringer solution and standardized by opacity. Equal amounts of suspension were then inoculated into each of the three vaccines. The mixtures were left at laboratory temperature, and samples were withdrawn at intervals and counted by the roll-tube or plate method. Tests with the sporing organisms were made in a similar manner, except that spore suspensions were used in which there were very few vegetative bacteria, or in which the vegetative bacteria had been killed by heat.

The technique used in the individual laboratories differed, however, in several respects. In one laboratory 10-ml. quantities of each vaccine were transferred to fresh sterile bottles and an inoculum of 0.1 ml. was added by a micrometer syringe; the exact quantity of the vaccine was thus known, and the dilution of the disinfectant was only 1%. In other laboratories the amount of vaccine in each bottle was not measured, so that minor variations from the supposed 25 ml. were almost certainly present, and the inoculum, which was added by a pipette or syringe, ranged from 0.25 to 0.5 ml., thus leading to a maximum dilution of the disinfectant of 2%. In three laboratories a viable count was made on the inoculum itself; in one laboratory the initial count was made on the vaccine immediately after the addition of the inoculum. The

number of organisms inoculated varied from a few thousand to several millions per ml. of vaccine. The subsequent counts were made at different times in different laboratories. In one laboratory one count was made after 2 hours and another after 24 hours; in the others several counts were made within the first few hours and one after 24 hours. The temperature to which the organisms were exposed varied in different laboratories.

Assessment of Results

With so many variations in technique in different laboratories analysis of the results obtained presented great difficulty. Reproduction of the individual protocols is impossible by reason of space. Preliminary examination of the data showed that in many of the experiments the number of the counts was too few or their distribution too uneven to justify comparison by means of the reaction velocity constant, or by the method recently advocated by Withell (1942) of estimating the time taken to destroy 50% of the organisms. On the whole the best method seemed to be to work out the geometric mean of each series of counts. In doing this the initial count and the final count, if all three vaccines were sterile, were omitted, and the arithmetic mean of the logarithms of the remaining counts was calculated. Table I illustrates the use of this method.

TABLE I.—Exposure of *Ps. pyocyanea* to Alcohol and to Phenol, showing Number of Organisms surviving after Different Times. Inoculum was 7,900 Organisms per ml. (Experiment 18 of Table II.)

Time in Minutes	25% Alcohol		0.5% Phenol		22.5% Alcohol	
	Nos.	Log. of Nos.	Nos.	Log. of Nos.	Nos.	Log. of Nos.
20	2,280	3.3579	6,500	3.8129	3,740	3.5729
40	372	2.5705	5,000	3.6990	2,240	3.3502
80	12	1.0792	2,500	3.3979	1,660	3.2201
135	3	0.4771	160	2.2041	1,460	3.1644
255	0	0.0000	0	0.0000	672	2.8274
390	0	0.0000	0	0.0000	56	1.7482
Total ..		7.4847		13.1139		17.8832
A.M. of logs ..		1.2475		2.1857		2.9805
G.M. ..		18 [†]		153		956

A.M. = Arithmetic mean. G.M. = Geometric mean. The 22.5% alcohol suspension was sterile after 24 hours.

The true sterilization time was not available, but the time at which the first sterile count was recorded was used as a rough index for comparing the activity of the three disinfectants in any one experiment.

Results

In none of the laboratories was evidence obtained to show that the strengths of the two disinfectants used had any destructive effect on the spores of *B. subtilis* or *Cl. sporogenes* within 24 hours. No further description of the results is therefore called for.

Twenty comparative experiments were made on *Staph. aureus*, *Bact. coli*, and *Ps. pyocyanea*. An illustrative protocol has already been given in Table I. A summary of all the experiments is provided by Table II. It will be seen that except for experiments 6 and 19, in which two or all three of the vaccines were sterile at the time of the first count, the geometric mean was least in the 25% and greatest in the 22.5% alcoholized vaccine, the count in the 0.5% phenolized vaccine occupying an intermediate position. Similarly in all the experiments in which the results could be compared, the 25% alcoholized vaccine became sterile first and the 22.5% alcoholized vaccine last. There seems, therefore, no reason to doubt the order of bactericidal potency of these three substances, though our data are not complete enough to enable us to state how much superior 25% alcohol is to 0.5% phenol, or 0.5% phenol is to 22.5% alcohol.

It will, however, be noted that even with very large inocula—far greater than would be likely to occur in practice as the result of using an imperfectly sterilized needle with which to withdraw vaccine from the bottle—there were only three experiments with the 22.5% alcoholized vaccine in which organisms were alive at the end of 24 hours. In experiments 4 and 11 the numbers of surviving organisms were 79 and 19

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per ml. respectively. In experiment 8, with an inoculum of 9,000,000 per ml., the 24-hour count in the 22.5% alcoholized vaccine was 198,000 and in the 0.5% phenolized vaccine 9,800 per ml. In experiment 5 a 24-hour count was not made, but as the count at $7\frac{1}{2}$ hours was only 8 per ml. there is every reason to believe that the vaccine would have been sterile within 24 hours.

With small inocula it is probable that sterility would be achieved with 22.5% alcohol in a time not very much longer than with 0.5% phenol. This supposition is supported by the finding of Dr. Carruthers at Cambridge that, with an initial inoculum of 80 to 150 organisms per ml., *Staph. aureus*, *Bact. coli*, and *Ps. pyocyanea* were destroyed by 25% alcohol within

between the two strengths of alcohol was remarkable. It has been demonstrated by Minervini (1898) that the most germicidal concentration of alcohol is between 50% and 70%. Above and below this level its effect is less. Judging from the findings of Tilley (1939) that in the 26% to 34% range a decrease of 2% in concentration approximately doubled the time necessary for sterilization of *Bact. typhosum*, and from our observations that a fall from 25% to 22.5% produced an even greater effect, it seems clear that the slope of the curve depicting the germicidal potency of alcohol in the 20% to 35% range of concentration must be fairly steep.

So far as it is permissible to compare the rate of destruction of *Staph. aureus*, *Bact. coli*, and *Ps. pyocyanea*, our results

TABLE II.—Exposure of Different Vegetative Organisms to Alcohol and to Phenol

Organism	No.	Laboratory	Approximate Temperature °C.	Inoculum : Organisms per ml. Vaccine	Geometric Means of Viable Counts per ml. Vaccine				Time of First Sterile Sample in Hours		
					No. of Counts	25% Alcohol	0.5% Phenol	22.5% Alcohol	25% Alcohol	0.5% Phenol	22.5% Alcohol
Staph. aureus	1	D	25-6	1,000	4	7	18	400	4	4	24
	2	D	23-5	1,500	5	21	40	214	4	4	24
	3	D	22-3	1,500	5	7	11	200	2	2	24
	4	A	20-2	7,100	6	304	1,235	5,811	7	24	>24
	5	B	—	13,000	6	10	86	380	2	4.5	>7.5
	6	C	23	232,000	1	0	0	50,500	2	2	24
	7	C	21-3	3,300,000	1	30	2,900	760,000	24	24	24
	8	C	23	9,000,000	2	202	278,000	1,235,000	24	>24	>24
Bact. coli	9	B	—	1,300	3	0	1	19	0.5	1	4
	10	D	22-3	3,000	4	3	5	868	1	2	24
	11	D	20-1	3,500	6	15	22	573	4	4	>24
	12	A	20-2	10,400	7	215	493	2,938	5	7.5	24
	13	C	21	298,000	1	0	1,010	1,030	2	24	24
	14	C	23	4,200,000	1	0	410	69,000	2	24	24
Ps. pyocyanea	15	D	22-3	1,000	1	0	19	104	0.5	1	1
	16	B	—	1,500	3	1	3	70	0.5	0.5	2
	17	D	20-1	2,500	3	21	126	538	4	4	4
	18	A	20-2	7,900	6	18	153	956	4	4	24
	19	C	21-3	189,000	1	0	0	0	2	2	2
	20	C	21-3	11,700,000	1	63	2,400	57,000	24	24	24

Note.—Owing to the differences in the size of the inoculum and other conditions the figures should be compared only horizontally. It should also be noted that the times given in the last three columns are not true "sterilization times."

15 minutes, by 0.5% phenol within 2 hours, and by 22.5% alcohol within 4 hours.

Laboratory C performed control experiments by inoculating along with each set of three vaccines a fourth bottle of heat-killed T.A.B.C. vaccine containing no disinfectant. In seven experiments the counts of the control vaccine at 2 and 24 hours did not differ from that of the inoculum by more than was to be expected from the sampling error of the technique, showing that no multiplication of the organisms had occurred. Even without any disinfectant, therefore, T.A.B.C. vaccine does not constitute a favourable medium for bacterial growth. The function of the added disinfectant is merely to deal with any organisms that may gain access to the bottle as the result of contamination. That 22.5% alcohol is capable of fulfilling this function there seems no reason to doubt.

Discussion

The experiments just described were undertaken in order to solve a specific practical problem, not to make an academic study of the reaction velocity and other constants of alcohol. For this reason only their general plan was laid down, each laboratory being left a considerable margin of freedom to decide on the detailed conduct of the experiments. That the four laboratories placed the three disinfectant solutions tested in the same order of potency is therefore all the more convincing.

The failure of alcohol to bring about any appreciable destruction of spores is in conformity with the observations of previous workers (for references see Coulthard and Sykes, 1936). Similarly, 0.5% phenol is known to be without effect on spores (Chick, 1908). Our results with spores therefore call for no comment.

With vegetative organisms, 22.5% and 25% alcohol and 0.5% phenol all proved to be moderately bactericidal. The order of potency, as determined by the four different laboratories using three different species of organism, was uniformly consistent, 25% alcohol proving the most and 22.5% alcohol proving the least bactericidal. The difference in killing power

showed little difference in the behaviour of these three organisms. Lockemann, Bär, and Totzeck (1941), on the other hand, found that *Staph. aureus* was considerably more resistant to all strengths of alcohol than either *Bact. coli* or *Ps. pyocyanea*.

Summary

Four laboratories carried out experiments in which the disinfectant action of 22.5% and 25% alcohol was compared with that of 0.5% phenol in T.A.B.C. vaccine.

A few observations made on the spores of *B. subtilis* and *Cl. sporogenes* failed to reveal any bactericidal activity of the three vaccines.

Eight experiments were carried out with *Staph. aureus*, six with *Bact. coli*, and six with *Ps. pyocyanea*, in which known numbers of organisms were inoculated into each of the three vaccines and in which the survivors were estimated at varying intervals of time by the roll-tube or plate method of counting.

In every experiment in which the figures were suitable for comparison it was found that the organisms perished most rapidly in the 25% and least rapidly in the 22.5% alcoholized vaccine, the 0.5% phenolized vaccine occupying an intermediate position in this respect.

Reasons are given for believing that 22.5% alcohol is only slightly inferior to 0.5% phenol in its germicidal potency, and that it is capable of destroying within a few hours vegetative bacteria that may gain access to T.A.B.C. vaccine by contamination.

There is no ground for the fear that the 22.5% alcohol used in Felix's new vaccine is likely to prove inefficient as a preservative.

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The Board of Education have recently issued a circular (1594) on the use of raw vegetables in school meals. They list the vegetables which can be used, briefly describe methods of preparing them, and give a few simple and useful recipes.