THE STERILIZATION OF T.A.B.C. VACCINE

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(With Plate III)

Introduction

The entire supply of T.A.B.C. vaccine for the Royal Navy is prepared at the Royal Naval Medical School, Greenwich; a "V" strain of *B. typhosus* is used. The method of sterilization employed is to heat the suspension at 58° C. for 1½ hours and then to add 0·5 per cent phenol; phenol in further amount to maintain this concentration is added after the subsequent dilution to give the requisite count in the finished vaccine. (In 1·0 c.c. 1000 million *B. typhosus* and 500 million each of *B. paratyphosus* A, B and C.) The high degree of immunity conferred by the vaccine is demonstrated by the returns for 1934, which show that in a total force of 83,000 men there were only five cases of typhoid and the same number of paratyphoid fever and that all of these cases recovered.

This vaccine must be exceedingly deficient in "Vi" antigen, for Felix (1934) has shown that when this substance is heated at 58° C. for 1½ hours it fails to produce Vi agglutinins in the rabbit, and (1935) that 0·5 per cent phenol alone, when used to sterilize *B. typhosus* suspensions, destroys the Vi antigen. It was to be expected, therefore, that if a method of sterilization could be devised which was without destructive action on the Vi antigen content it would be possible to prepare a vaccine of enhanced immunological quality.

In investigating this problem it was postulated that practical considerations demanded that the vaccine should:

(*a*) Not require a dose in excess of 1·0 c.c. (to avoid the introduction of a concentration process the method of sterilization therefore must be capable of killing in suspensions bacteria numerically not less than 2500×10^6 per c.c.

(*b*) Retain its potency for at least 6 months.

(*c*) Have antiseptic properties capable of preventing secondary growth.

(*d*) Contain nothing injurious to human tissues when injected subcutaneously.

Heat treatment

In view of the finding by Horgan (1936) that suspensions of *B. typhosus* in concentrations of 1000×10^6 per c.c. could be sterilized by a temperature of 55° C. for 1 hour, and that this treatment has no detrimental effect on the Vi antigen, experiments were made to determine whether higher concentrations could be sterilized by the same means.

Twenty-four-hour old cultures on beef bouillon agar were washed off, suspended in 0·85 per cent saline *p*H 7·4 and standardized by opacity. The

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suspensions tested ranged from 10×10^9 down to 1×10^9 per c.c. 2·0 c.c. of each suspension were measured into a Wassermann tube and immersed in a water-bath at 55° C. so that the level of the water in the bath was ½ in. higher than the surface of the suspension. After 1 hour sterility tests were carried out by inoculating some of the suspension from each tube into broth. These tests were repeated after a further period of half an hour. The temperature of the bath was checked by three thermometers (one N.P.L. standardized). The cultures tested in this manner included three V strains of *B. typhosus*, Ty 2, Giglioli, and Rawlings Rejuvenated (Perry) and three strains of *B. para-typhosus*, A (Mears), B (Rowland) and C (Hirchfield). It was found that none of these cultures was sterilized by this means. The tests were repeated several times but always with the same result. The discrepancy between these findings and those of Horgan is probably due to some unknown factor which renders certain strains of *B. typhosus* either more resistant or more susceptible to the effects of heat.

Many difficulties closely resembling those of the present problem are met with in the preparation of an effective anti-plague vaccine. In the *Haffkine Institute Report*, 1932–5, it is stated that sterilization of this vaccine by heating for 15 min. at 55° C. instead of at 60° C. for 1 hour greatly enhanced its immunological properties. This tends to confirm the suggestion of Felix (1934) that antigens with activities similar to those of Vi antigen are to be found in bacteria other than *B. typhosus*, while Horgan's work appears to indicate that these unstable antigens are not materially affected by heating at 55° C. It was thought, therefore, that a potent Vi antigen typhoid vaccine might be prepared if the factors governing the character of heat sensitiveness of the typhoid group of bacteria could be discovered, but this line of research was not pursued as an alternative method of sterilization promised to offer a more immediate solution to the problem.

This method is a proprietary one called "Katadyne", and its effects are attributed to the oligodynamic action of minute amounts of silver which are imparted to the material treated.

Katadyne as a sterilizing agent

Experiment

An 18-hour-old culture of *B. typhosus* strain Ty 2 grown on beef bouillon agar *p*H 7·4 was washed off and suspended in normal saline *p*H 7·4. The opacity of this suspension was equivalent to 6×10^9 per c.c. Katadyne beads, 25 c.c., were introduced into 100 c.c. of this suspension contained in a sterile screw-cap bottle which was then placed in a refrigerator at a temperature of 4° C. Sterility tests were made daily by inoculating some of the suspension into broth and incubating at 37° for 7 days. After 9 days' storage in the refrigerator the suspension was sterile. It was then decanted into another sterile screw-cap bottle, and further tests were made in broth and glucose agar

which confirmed the sterility. This suspension will be designated as "K 1". Table I shows the reactions obtained when this suspension was tested against Vi, O and H sera. The Vi serum A was prepared in accordance with the recommendation of Felix (1934) by immunizing a rabbit by four intravenous injections of a living culture of *B. typhosus* (V strain Giglioli), 1 week after the last injection the rabbit being bled and the serum separated, and stored without preservative. After absorption with the highly agglutinable strain Ty H 901 this serum had a Vi titre of 1/640 with the V strain Watson and 1/320 with Ty 2. The O serum was prepared by immunizing a rabbit with a heat-killed culture of Ty O 901 grown on phenol agar; this was also stored without preservative.

Table I. *Demonstrating the agglutination to Vi sera and the resistance to O antibody of Ty 2 suspension K 1 sterilized by katadyne*

Serum	Dilution	Ty 2 (live)	Watson (live)	K 1	K 1 heated at 100° C. for 15 min.	30 min.	60 min.	Ty O (Oxford)	Ty H 901
Vi serum (rabbit A) after absorption with Ty H 901 (live)	1 : 20	+++	+++	+++	(±)	(±)	(±)	(±)	(±)
	1 : 40	+++	+++	+++	−	−	−	−	−
	1 : 80	+++	+++	+++	−	−	−	−	−
	1 : 160	+++	+++	+++	−	−	−	−	−
	1 : 320	+	++	+++	−	−	−	−	−
	1 : 640	−	++	++	−	−	−	−	−
	1 : 1280	−	−	++	−	−	−	−	−
	1 : 2500	−	−	−	−	−	−	−	−
Pure O serum (rabbit B)	1 : 250	(±)	Not	(±)	+++	+++	+++	+++	Not
	1 : 500	−	tested	(±)	+++	+++	+++	+++	tested
	1 : 1000	−	,,	(±)	+++	+++	+++	+++	,,
	1 : 2000	−	,,	−	++	++	+++	+++	,,
	1 : 4000	−	,,	−	(±)	(±)	++	+++	,,
	1 : 8000	−	,,	−	−	−	+	++	,,
	1 : 16,000	−	,,	−	−	−	(±)	+	,,
	1 : 20,000	−	,,	−	−	−	−	(±)	,,
	1 : 40,000	−	,,	−	−	−	−	−	,,
H serum (Oxford), titre 1/250	1 : 250	++	++	++	Not tested	Not tested	Not tested	Not tested	++

As the effect of katadyne on O antigen was unknown, it was necessary to distinguish between either true resistance to O antibody caused by the presence of Vi antigen, or absence of agglutination due to destruction of O agglutinogen by katadyne. Some of suspension K 1 was therefore heated at 100° C. and again tested against these sera. The results (Table I) showed that there was a true resistance to O antibody which was removed by boiling the suspension, and that katadyne had had no destructive action on O agglutinogen.

Further experiments with katadyne

In the course of these experiments it was found that the katadyne elements became inert after remaining in saline for 7 days. This was apparently due to the formation of an insoluble outer coating. By replacing these elements with fresh ones at weekly intervals it was possible to sterilize suspensions of 15×10^9 per c.c., but very heavy suspensions such as 25×10^9 per c.c. could not be sterilized even with three or four replacements. The sterilization time was

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variable. With weak suspensions (3×10^9 per c.c.) it took from 9–12 days and with heavier suspensions even longer. The results of experiments with four suspensions K 1, K 2, K 3, K 4, illustrate this (see Table II), and appear to indicate that this silver preparation is only sufficiently soluble in saline to sterilize a limited number of organisms.

Table II. *Showing the limitations of katadyne as a sterilizing agent for* B. typhosus *vaccine*

Emulsion	No. of organisms per c.c.	No. of replacements of fresh katadyne elements at weekly intervals	Result
K 1	6×10^9	None	Sterile after 9 days' treatment
K 2	15×10^9	One	Sterile after 10 days' treatment
K 3	22×10^9	Four	Alive after 35 days' treatment
K 4	3×10^9	None	Sterile after 9 days' treatment

For comparison the reactions obtained with the suspensions K 1, K 2 and K 4 when tested against Vi, O and H sera are shown in Table III.

Tables I and III show that sterilization of Ty 2 suspensions by katadyne produced increased sensitiveness to Vi antibody, and also a slight reduction

Table III. *Showing agglutination reactions of various emulsions of Ty* 2 *sterilized by katadyne to Vi, O, H anti-sera*

Serum	Dilution	K 1	K 2	K 4	Ty 2 (live)
Pure Vi serum (rabbit A), titre 1/640 to Watson	1 : 40	+ + +	+ + +	+ + +	+ + +
	1 : 80	+ + +	+ + +	+ + +	+ + +
	1 : 160	+ + +	+ + +	+ + +	+ + +
	1 : 320	+ + +	+ + +	+ + +	+
	1 : 640	+ + +	+ + +	+ +	–
	1 : 1280	+ +	+ +	+ +	–
	1 : 2560	–	–	–	–
O serum (rabbit B), titre 1/10,000 to Ty O 901	1 : 100	(±)	(±)	(±)	–
	1 : 200	(±)	(±)	(±)	–
	1 : 400	(±)	–	(±)	–
	1 : 800	(±)	–	(±)	–
	1 : 1600	–	–	–	–
	1 : 3200	–	–	–	–
	1 : 6400	–	–	–	–
	1 : 12,800	–	–	–	–
H serum (Oxford), titre 1/250	1 : 250	+ +	+ +	+ +	+ +

of resistance to O antibody; these effects point to some loss of Vi antigen (Felix, 1934). Although these tests for Vi antigen carried out *in vitro* give no real indication of the true immunogenic properties of katadyne sterilized suspensions, they were considered sufficiently promising to justify further investigation in that direction.

THE IMMUNOGENIC QUALITIES OF KATADYNE STERILIZED SUSPENSIONS

For this investigation a number of rabbits were immunized as follows:

Using live virulent suspensions of Ty 2	Rabbit No. 3	Horgan's method (1936)
	Rabbit No. 6	Felix's method (1934)
Using K 2 suspensions	Rabbit No. 2	Four intravenous injections at 2-day intervals of 500, 1000, 2000, 2000 million
	Rabbit No. 7	Horgan's method (1936). (Same dosage and method as rabbit No. 3)
Using freshly prepared suspensions of Ty 2 sterilized by heating at 58° C. for 1½ hours, with subsequent addition of 0·5 % phenol	Rabbit No. 4	Same method and dosage as rabbit No. 2
Using freshly prepared suspensions of Ty H 901 sterilized by heating at 58° C. for 1½ hours with subsequent addition of 0·5 % phenol	Rabbit No. 5	Same method and dosage as rabbits Nos. 2 and 4

One week after the last inoculation blood was drawn off from the heart of each rabbit and the serum separated and stored without preservative.

The titres of these sera are shown in Table IV.

Table IV. *Showing the agglutinogenic properties of katadyne sterilized suspension compared with those of live suspensions and those sterilized by heat and phenol*

Rabbit No.	Emulsion used for immunization	Dose in millions	Titres of antibodies present in the sera Vi: H	O	Watson (live)	Ty 2 (live)
3	Ty 2 (live)	1950	40,000	5000	320	160
6	Ty 2 (live)	800	10,000	2500	320	160
2	K 2 = Ty 2 sterilized by katadyne	5500	5000	1280	160	80
7	K 2 = Ty 2 sterilized by katadyne	1950	5000	5000	320	80
4	Ty 2 sterilized by heating at 58° C. for 1½ hours + 0·5 % phenol	5500	5000	5000	0	0
5	Ty H 901 sterilized by heating at 58° C. for 1½ hours + 0·5 % phenol	5500	5000	5000	0	0

Table IV also shows that the anti-Vi titre of rabbit No. 7 serum (obtained by immunization with a katadyne sterilized suspension) compares favourably with that of rabbit No. 3 serum (obtained by immunization with living cultures). But as the mere presence of Vi agglutinins in a serum is no indication of its protective properties (Felix (1935) produced Vi agglutinins in rabbits, using formalin sterilized suspensions, but found that these sera failed to confer passive immunity against infection from V strains) it was necessary to test the protective value of these sera before the immunological properties of katadyne sterilized suspensions could be assessed. For this reason it was decided to compare the protective values of sera 3, 4, 5 and 7 by means of passive immunization of mice.

The sterility of these sera was first confirmed and then they were injected intramuscularly into mice weighing from 25–30 g.; 24 hours later a test dose of a living suspension of Ty 2 (3 × M.L.D.) was injected intraperitoneally.

Table V shows that this experiment was of little value as each group of mice, with the exception of that group injected with serum No. 5, showed 100 per cent survival. The experiment was therefore repeated, using a much smaller dose of each serum but the result was practically the same, as will be seen in Table VI.

Table V. *Passive immunization of mice*

Serum	Serum dose in c.c.	Result
Rabbit No. 3 immunized with living suspensions of Ty 2	1·0	4/4
	0·5	4/4
Rabbit No. 4 immunized with suspension of Ty 2, sterilized by heating at 58° C. for 1½ hours + 0·5 % phenol	1·0	4/4
	0·5	4/4
Rabbit No. 5 immunized with suspensions of Ty H 901, sterilized by heating at 58° C. for 1½ hours + 0·5 % phenol	1·0	4/4
	0·5	2/4
Rabbit No. 7 immunized with K 2 suspension, i.e. suspension of Ty 2 sterilized by katadyne	1·0	4/4
	0·5	4/4
Controls:		
1/3 test dose of living suspension of Ty 2 = 50×10^6		0/8
1 „ „ = 150×10^6		0/4

Denominator = no. of mice tested.
Numerator = no. of mice surviving.

Table VI

Serum	Dose in c.c.	Result
Rabbit No. 3	0·1	10/10
Rabbit No. 4	0·1	9/10
Rabbit No. 7	0·1	9/10
Controls:		
1/2 test dose = 75×10^6 living suspension Ty 2		0/4
1/3 test dose = 50×10^6 living suspension Ty 2		0/4

Numerator = no. of survivors.
Denominator = no. of mice tested.

As Felix (1935) found that serum produced by the immunization of rabbits with phenolized suspensions of *B. typhosus* failed to protect mice against a 3 × M.L.D. of a V strain of *B. typhosus*, even when given in such large doses as 1·0 c.c., these results were unexpected and it was decided before proceeding further to investigate another form of katadyne, electro-katadyne.

Experiments with electro-katadyne

These were performed with a small pocket apparatus consisting of a dry battery connected to two silver electrodes (Plate III). When the two electrodes are immersed in water small amounts of silver are given off into the water; this silver is in the form of an unstable compound which ionizes freely. The precise chemical nature of this compound is not known. The apparatus described cannot be used in strong solutions such as 0·85 per cent saline or in distilled water, for in the former case the electrodes become polarized, and in the latter the current is not conducted.

Experiment

Using the V strain Ty 2, an 18-hour growth on beef bouillon agar was washed off, and suspended in distilled water. This suspension was standardized by opacity to 6×10^9 per c.c. and was treated with electro-katadyne by immersing the two electrodes of the apparatus in 50 c.c. for 30 min. It was then

allowed to stand for 4 hours at room temperature, and was then transferred to a sterile screw-cap bottle containing 0·45 g. of sodium chloride. It was shaken well to dissolve the salt. Sterility tests were made by inoculating the treated suspension into tubes of broth and glucose agar which were incubated for one week at 37° C. These tests showed the suspension to be sterile.

It is necessary to explain that this suspension was greatly overdosed with electro-katadyne, as it was discovered later that a dosage equivalent to 1/10 of that used in the above experiment was more than sufficient to render such a weight of suspension sterile; this suspension was so over-dosed that it turned black when exposed to light. In practice it would be inadvisable and unnecessary to immerse the electrodes directly in the suspension, for it was found that suspensions could be equally quickly sterilized by treating the water first and suspending the organisms in the treated water. When this suspension was being treated the current passing approximated 7 mA., equivalent to a dosage of 0·014 per cent silver (see p. 549).

Table VII shows the reactions of this suspension (EC 1) to pure Vi, O and H sera.

Table VII. *Showing the agglutination reactions of an electro-katadyne sterilized suspension of a V strain of* B. typhosus

Serum	Dilution	Watson (live)	Ty 2 (live)	EC 1	EC 1 boiled for 1 hour	Ty 901
Pure Vi sera (rabbit No. 6), after absorption with Ty H 901 emulsion	1 : 40	+ + +	+ + +	+ + +	−	−
	1 : 80	+ + +	+ + +	+ + +	−	−
	1 : 160	+ + +	+ +	+ + +	−	−
	1 : 320	+	−	+ +	−	−
	1 : 640	−	−	(±)	−	−
Pure O serum (rabbit B), titre 1/10,000	1 : 40	(±)	(±)	+	+ + +	+ + +
	1 : 80	−	−	(±)	+ + +	+ + +
	1 : 160	−	−	−	+ + +	+ + +
	1 : 320	−	−	−	+ + +	+ + +
	1 : 640	−	−	−	+ + +	+ + +
	1 : 1280	−	−	−	+ +	+ + +
	1 : 2560	−	−	−	+ +	+ + +
	1 : 5000	−	−	−	+	+ +
	1 : 10,000	−	−	−	−	+
Pure H serum (Oxford), titre 1/5000	1 : 40	+ + +	+ + +	+ + +	−	+ + +
	1 : 80	+ + +	+ + +	+ + +	−	+ + +
	1 : 160	+ + +	+ + +	+ + +	−	+ + +
	1 : 520	+ + +	+ + +	+ +	−	+ + +
	1 : 640	+ + +	+ + +	+ +	−	+ + +
	1 : 1280	+	+ +	(±)	−	+ +
	1 : 2500	(±)	+	−	−	+ +
	1 : 5000	(±)	(±)	−	−	+

This table shows that the agglutination reactions obtained with an electro-katadyne sterilized suspension of a V strain of *B. typhosus* differ hardly at all from those obtained with living suspensions.

THE IMMUNOGENIC AND AGGLUTINOGENIC PROPERTIES OF ELECTRO-KATADYNE STERILIZED SUSPENSIONS OF V STRAINS OF *B. TYPHOSUS*

Felix (1936*a*) has stated that the treatment of suspensions of Vi antigen containing bacilli with alcohol has no detrimental effect on the immunogenic activities of the Vi antigen. It was therefore considered that in order to

ascertain the value of an electro-katadyne sterilized suspension as an immunogenic agent it would be necessary to compare it both with a suspension heated at 58° C. for 1½ hours with subsequent addition of 0·50 per cent phenol, and with one sterilized by Felix's (1936*b*) method using 96 per cent alcohol. Therefore, each of these three suspensions of the V strain Ty 2 was used to immunize rabbits by giving three intravenous doses of 500, 1000 and 2000 million with a 4 days' interval between each dose. One week after the last injection each rabbit was bled from the heart and the serum separated. To ensure sterility these three sera were filtered through a Seitz E.K. filter, and stored without preservative.

The Vi, O and H titres of these sera are shown in Table VIII.

Table VIII. *Showing the agglutinogenic properties of an electro-katadyne sterilized suspension compared with that sterilized by alcohol and that sterilized by heating at* 58° C. *for* 1½ *hours* + 0·5 *per cent phenol*

Rabbit No.	Suspension used for immunization	Dose in millions	H	O	Vi (Ty 2 living)
9	Ty 2 sterilized by electro-katadyne, i.e. EC 1	3500	10,000	5000	320
10	Ty 2 sterilized by heating at 58° C. for 1½ hours and subsequent addition of 0·5% phenol	3500	10,000	5000	0
11	Ty 2 sterilized by alcohol 96% (Felix, 1936*b*)	3500	0	5000	160

A COMPARISON BY PASSIVE IMMUNIZATION EXPERIMENTS OF THE PROTECTIVE VALUE OF VARIOUSLY PRODUCED ANTI-TYPHOID SERA

It has been shown earlier that passive immunization experiments with mice failed to demonstrate the different protective properties of variously produced anti-typhoid sera because the test dose of the infecting organism was too small to allow any comparison to be made. Before proceeding to carry out similar experiments for estimating the protective properties of a serum produced by immunization with electro-katadyne sterilized suspensions of *B. typhosus*, and in order to ensure that these experiments should have some comparative value, it was therefore necessary to discover what test dose should be employed. For these reasons the following preliminary experiment was made.

A number of mice each weighing between 25–30 g. was divided into three groups. Each mouse in the first group received an injection of 0·5 c.c. of serum No. 9, the second group 1·0 c.c. of serum No. 10, and the remaining group 0·5 c.c. of this serum. Twenty-four hours later a living suspension of the virulent strain Ty 2 of *B. typhosus* in saline was injected intraperitoneally in doses varying from 250×10^6 to 600×10^6, i.e. five to twelve times the M.L.D. In each case the test dose was administered in a total volume of 0·5 c.c.

The mice were then kept under observation for one week. The results are shown in Table IX.

A comparison of the protective values of the three differently produced sera Nos. 9, 10, and 11 with that of serum No. 3 which had been produced by

immunizing a rabbit with living suspensions of the strain Ty 2, was now carried out. In view of the findings of the previous experiment it was decided that for this experiment a suitable test dose to employ would be $10 \times$ M.L.D., i.e. 500×10^6.

The sterility of each batch of serum having been first confirmed, mice in groups of ten were injected with these sera, and 24 hours later the test dose of a living suspension of the strain Ty 2 in saline, made from an 18-hour-old agar culture and contained in a total volume of 0·5 c.c. was injected intraperitoneally. For a control twelve mice which had not received an injection of serum were injected intraperitoneally, six receiving 0·5 c.c. of the test dose suspension diluted 1/10 with saline and six the same amount diluted 1/5. These mice were then kept under observation for 1 week.

Table IX

Serum	Dose of sera in c.c.	Test dose of live suspension of Ty 2 in millions: 50	250	300	350	400	450	500	550	600
Rabbit No. 9 immunized with suspension of Ty 2, sterilized with electro-katadyne, i.e. EC 1	0·5	—	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
Rabbit No. 10 immunized with Ty 2 suspension, sterilized by heating for 1½ hours at 58° C. +0·5 % phenol	1·0	—	1/1	1/1	1/1	1/1	1/1	1/1	0/1	1/1
	0·5	—	1/1	1/1	0/1	0/1	1/1	0/1	1/1	0/1
Control	0·0	0/2	—	—	—	—	—	—	—	—

The denominator = no. of mice tested
The numerator = no. of mice surviving

Table X. *Showing the comparative protective values of variously produced anti-typhoid sera*

Serum	Dose of sera in c.c.	Result
Rabbit No. 3 immunized with living suspension of BT Ty 2	0·5	8/10
	0·25	10/10
Rabbit No. 9 immunized with a suspension of BT Ty 2, sterilized with electro-katadyne, i.e. EC 1	0·5	10/10
	0·25	9/10
Rabbit No. 10 immunized with a suspension of BT Ty 2, sterilized by heating at 58° C. for 1½ hours + 0·5 % phenol	1·0	4/10
	0·5	1/10
	0·25	2/10
Rabbit No. 11 immunized with a suspension of BT Ty 2, sterilized by treatment with 96 % alcohol	0·5	8/10
	0·25	6/10
Controls: 1/10 test dose = 50×10^6	Living suspension of Ty 2	0/6
1/5 test dose = 100×10^6		0/6

Test dose = $500 \times 10^6 = 10$ M.L.D. Denominator = no. of mice tested
Numerator = no. of survivors

The results of this experiment are shown in Table X and are so self-evident that they do not require to be elaborated. The high degree of protection afforded by serum No. 9 indicates that in electro-katadyne a means is available for sterilizing *B. typhosus* vaccine which is free from the disadvantages of such processes as have been employed hitherto.

The bactericidal properties of electro-katadyne in water

The bactericidal properties of water treated with electro-katadyne depend upon a number of factors, the most important of which are the *p*H, the presence of other ionizable chemical substances, the temperature, and the amount of ionizable silver which has passed into solution.

No attempt has yet been made to estimate the precise quantity of silver necessary for the sterilization of live suspensions of *B. typhosus*. But in the commercial literature it has been stated that the quantity of silver entering any solution treated with electro-katadyne bears a constant ratio to the amount of current passing through the solution during treatment. In the course of experiments it has been found that the weight of silver in a treated water was equivalent to 2·0 mg. per hour per milliampere of current passing through the water, but it is to be expected that the quantity of silver entering into water so treated will vary enormously with the physical and chemical character of that water. For example with London tap water, when using the apparatus already described, it was found that a current of 7 mA. passed through the water but with an artificially made water the current only reached a maximum of 1 mA. In order, therefore, to obtain consistent and comparable results a specially prepared artificial water was employed in all the following experiments. This water consisted of:

Sodium chloride 0·066 g.
Distilled water 1000 c.c. } = Chlorides 4·0 parts per 100,000.

The *p*H was adjusted to 7·6 with 14·0 c.c. of *M*/100 sodium carbonate per litre. This solution allowed a current of 1 mA. to pass.

Experiments to discover the maximum bactericidal property of electro-katadyne for the sterilization of *B. typhosus* suspensions

Experiment

The solutions employed were quantities of:

50 c.c. of artificial water treated with electro-katadyne for A 5, B 10, C 15 and D 20 min. respectively.

A 24-hour-old culture of *B. typhosus* strain Ty 2 grown on beef bouillon agar was washed off, and suspended in sterile artificial water. This suspension was standardized by opacity to 10×10^9 per c.c. 2·0 c.c. of this suspension was then added to 2·0 c.c. of solutions A, B, C and D in sterile Wassermann tubes which were then placed in a water bath at 37° C. Sterility tests were made hourly for a period of 4 hours by inoculating the suspension from each tube into broth and incubating for 1 week at 37° C. The results of this experiment are shown in Table XI.

It will be seen that the "artificial water" treated for 20 min. with electro-

katadyne and diluted half was more than sufficient to sterilize a suspension of 5×10^9 per c.c. in 4 hours.

The maximum weight of suspension that could be sterilized by such a solution was now ascertained.

Table XI. *Bactericidal properties of electro-katadyne in artificial water*

Solution	No. of min. for which artificial water was treated with electro-katadyne	Approximate % silver present in solution	No. of hrs. *B. typhosus* suspension was exposed to electro-katadyne solution			
			1	2	3	4
A	5	0·00016	+	+	+	+
B	10	0·00033	+	+	+	−
C	15	0·0005	+	+	−	−
D	20	0·00066	−	−	−	−

+ =growth in test broth
− =test broth sterile after 7 days' incubation at 37° C.

For this purpose another experiment similar to that just described was carried out, using only solution D with a number of suspensions of *B. typhosus* which varied from 20×10^9 per c.c. down to $7{\cdot}5 \times 10^9$ per c.c. The results are appended in Table XII.

Table XII. *Showing the limitations of electro-katadyne as a sterilizing agent for* B. typhosus

Exposure time in hours	Approximate % of silver	Suspension of *B. typhosus* 10^9 per c.c. in artificial water			
		7·5	10	15	20
1	0·00066	+	+	+	+
2	0·00066	−	+	+	+
3	0·00066	−	+	+	+

+ =growth in test broth
− =no growth in test broth after 7 days' incubation at 37° C.

It was found that suspensions sterilized with electro-katadyne treated water as described above and after salt had been added gave reactions to Vi, O and H sera similar in every respect to those obtained with suspension EC 1 (Table VII).

THE BACTERICIDAL PROPERTIES OF ELECTRO-KATADYNE IN SALINE

The necessity for a vaccine to have sufficient antiseptic qualities to prevent it from becoming easily contaminated has been referred to already. It was thought that owing to the high concentration of sodium chloride in the finished vaccine (0·85 per cent) the bactericidal properties of electro-katadyne in such a solution might be so reduced as to be of no practical value. The following experiment showed that this was not the case.

Experiment

50 c.c. of sterile artificial water was treated with electro-katadyne for 30 min. and then added to 0·45 g. of sodium chloride in a screw-cap bottle previously sterilized in the autoclave. After being well shaken to dissolve the salt a series of different dilutions of this solution was made, using sterile 0·85 per cent saline as the diluent. 10 c.c. of each dilution was then contaminated with 0·1 c.c. of a 24-hour-old living broth culture of *B. typhosus*; one tube of 10·0 c.c. of 0·85 per cent saline was treated similarly to act as a control.

A similar experiment was made, using a living 24-hour broth culture of staphylococcus as the contaminant. After being contaminated these tubes were allowed to stand at room temperature, and sterility tests were made daily for a period of 12 days by inoculating some of the solution from each tube into broth which was incubated for 7 days at 37° C.

Table XIII. *Bactericidal properties of electro-katadyne in 0·85 per cent saline*

Day of test dating from time of contamination	Contaminating organism	Electro-katadyne treated artificial water +0·85% sodium chloride: Undiluted	Diluted with normal saline: 1/2	1/4	1/8	1/16	Saline control
1st day	*Staphylococcus*	+	+	+	+	+	+
	B. typhosus	+	+	+	+	+	+
2nd day	*Staphylococcus*	+	+	+	+	+	+
	B. typhosus	−	−	−	−	+	+
3rd, 4th, 5th, 6th and 7th days	*Staphylococcus*	−	+	+	+	+	+
	B. typhosus	−	−	−	−	+	+
8th, 9th, 10th and 11th days	*Staphylococcus*	−	+	+	+	+	+
	B. typhosus	−	−	−	−	−	+
12th day	*Staphylococcus*	−	−	+	+	+	+
	B. typhosus	−	−	−	−	−	+
Approximate % of silver present		0·002	0·001	0·0005	0·00025	0·000125	0

+ =growth in test broth.
− =no growth in test broth after 7 days at 37° C.

The results of these tests are shown in Table XIII, and indicate that if a vaccine contained 0·002 per cent of electro-katadyne silver, this quantity would be sufficient to maintain its sterility against the usual risks of contamination; it is unlikely that the amount of contamination occurring in practice would with ordinary care ever reach such an excessive degree as that employed in these experiments. The amount of silver is so minute that it may be accepted that it would not render a vaccine injurious to human tissue if injected subcutaneously in 1·0 c.c. doses.

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THE STABILITY OF ELECTRO-KATADYNE STERILIZED SUSPENSIONS OF V STRAIN OF *B. TYPHOSUS*

It will, of course, be evident that it is too early to report decisively on the general application of the electro-katadyne process of sterilization of *B. typhosus* suspensions, as sufficient time has not elapsed to determine the stability of vaccines in the production of which this process has been employed. Investigations regarding the permanence of the immunogenic properties of this new vaccine are being continued.

SUMMARY

1. Horgan's (1936) findings with regard to heat sterilization of *B. typhosus* vaccine are not confirmed.

2. Silver in the form of katadyne or electro-katadyne appears to be an effective sterilizing agent for *B. typhosus* suspensions.

3. Katadyne and electro-katadyne have no destructive action on Vi, O or H agglutinogens of *B. typhosus*.

4. Electro-katadyne, even when used in excessive doses, has no detrimental effect on the immunizing activities of Vi antigen.

5. Electro-katadyne sterilized suspensions of V strains of *B. typhosus* are more effective immunogenic agents than those sterilized by heat and phenol.

6. The bactericidal properties of electro-katadyne, though impaired in 0·85 per cent saline, are adequate as a means for preserving vaccine.

7. It is suggested that katadyne sterilized suspensions of V strains might be of value in testing for the presence of Vi agglutinins in human sera since they are hypersensitive to Vi antibody while retaining most of their resistance to O antibody.

8. In view of the suggestion by Felix that additional antigens with activities similar to those of Vi antigen may exist in bacteria other than *B. typhosus*, it may be found that the electro-katadyne process has an application in the preparation of vaccines generally, particularly those which have hitherto proved ineffectual in prophylaxis.

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