THE STERILIZATION OF LIPOVACCINES.

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The proposal to substitute suspensions of bacterial bodies in oil for suspensions in saline solution as a vaccine for use in combating typhoid fever and pneumonia theoretically offers great advantages. Provided the vaccination is safe and efficient, it would be of great importance to be able to get the same result with a single application that has, in the past, been accomplished with three doses of the prophylactic.

The evidence so far at hand would seem to show that the vaccination with oily suspensions causes a formation of antibodies comparable with that afforded by suspensions in water, and thus renders it probable that a similar degree of protection may be given by the lipovaccine. The matter is, however, of sufficient importance to justify repeated examination.

When material of this nature is to be widely distributed its sterility should be assured both by the method of preparation and control tests on the finished product. In this respect the methods proposed by Whitmore and Fennel[1] are not altogether satisfactory. The procedures recommended afford many opportunities for contamination, the oil interferes with the action of antiseptics so that these cannot be depended upon for a final sterilization, and lastly it is far from easy to make satisfactory tests for sterility on the finished product.

Rosenow and Osterberg,[2] recognizing these difficulties, propose to kill the bacteria and any contaminating organisms with a water solution of an antiseptic, to form an emulsion with the oil, and finally

[1] Whitmore, E. R., and Fennel, E. A., *J. Am. Med. Assn.*, 1918, lxx, 902.

[2] Rosenow, E. C., and Osterberg, A. E., *J. Am. Med. Assn.*, 1919, lxxiii, 87.

to remove the water by vacuum distillation at a comparatively low temperature. This appears from their statement to be an efficient method of preparation.

In approaching this matter we have followed another line of thought and wish to record our results at this time as a contribution to the subject without, however, attempting to decide which method may in the end be best.

Loeffler[3] has stated that by the application of dry heat bacteria may be killed without destroying their antigenic properties. The temperature he used was 70°C. and the exposure was prolonged for days or weeks. It is known that glassware and fabrics may be sterilized by exposure to dry heat for a number of hours, the length of exposure varying inversely with the temperature. Thus 130°C. for 3 hours is as efficient as 160°C. for 1 hour. The suspensions of bacteria in oil are essentially dry preparations, and it seemed possible that the finished vaccine could be sterilized at an intermediate temperature without destroying its antigenic qualities. As a matter of fact, our results indicate that this may be accomplished with pneumococcus. We have so far been unsuccessful with *Bacillus typhosus*.

EXPERIMENTAL.

Pneumococcus lipovaccine was prepared according to the method of Whitmore and Fennel.[1] Briefly, the bacteria were grown in glucose broth and separated by centrifugalization after about 18 hours growth. The bacterial mass was dried in an oven at 53°C. over lime. The dry mass was ground in a Pyrex jar with steel balls for a number of hours, a mixture of anhydrous lanolin and cottonseed oil (Wesson) added, and the grinding continued. Finally, sterile cottonseed oil containing 0.25 per cent chloretone was added in such amount that each cubic centimeter of the finished product would contain 2 mg. of the dried bacterial powder. The grinding was then continued for a number of hours, the product put into suitable bottles or ampules and sealed as ready for distribution.

Control tests for sterility revealed the presence of *Bacillus subtilis* and sometimes other bacteria in the dried mass in a majority of

[3] Loeffler, F., *Deutsch. med. Woch.*, 1913, xxxix, 1025.

instances. When the dried bacterial mass was clean the finished product was nearly always sterile. The pneumococcus does not survive the drying process. If the dried mass containing *Bacillus subtilis* is ground in oil containing chloretone this contaminating organism will remain viable for months. With experience in manipulation the amount of material lost because of contamination was reduced, but even with the greatest care it always remained high.

In an attempt to overcome this difficulty we exposed the finished vaccine to a temperature of 130°C. for 3 hours in an electric oven (Freas), and to 120°C. for 12 hours, temperatures which are sufficiently high to sterilize any material. The vaccine, heated and unheated, was administered to healthy mice (0.5 cc. subcutaneously), and after varying periods the resistance of the mice to pneumococcus infection was tested by intraperitoneal injection of doses of culture known to be fatal, or multiples thereof. The results of two experiments are given in Tables I and II.

The experiments show that a considerable degree of protection is afforded to mice by the administration of pneumococcus lipovaccine. In the occasional instances in which the vaccinated mice died it was found that the vaccine had not been absorbed, almost the whole mass injected remaining in the subcutaneous tissues. Protection is exerted against at least ten fatal doses of culture. The mice in the experiments reported had been treated with vaccine 35 and 38 days previously. In other experiments in which the interval was 21, 56, and 110 days, there was no evidence of protection, indicating that the immunity following a single dose of lipovaccine is slow to develop and is transient. The heat treatment did not decrease the antigenic qualities of the vaccine appreciably.

The administration of vaccine in practice as a prophylactic against pneumonia is still in the stage of trial. Since at present the qualities of typhoid vaccine are of much greater interest, we have extended our work to include this phase of the subject.

Typhoid lipovaccine[4] similarly heated and unheated was administered to rabbits intraperitoneally, a single dose of 1 cc. being given. The blood was tested for its agglutinin content at intervals there-

[4] The vaccines used in this experiment were obtained from the U. S. Army Medical School through the courtesy of Major H. J. Nichols.

TABLE I.

Protection Test of Mice Treated with Pneumococcus Lipovaccine.

The vaccinating dose was administered 35 days before the test. The test consisted of inoculation with the indicated quantity of a 24 hour bouillon culture injected intraperitoneally with normal salt solution sufficient to make a total volume of 0.5 cc.

Vaccine.	Culture.		Result.			
	Type.	Dilution.	After 24 hrs.	After 48 hrs.	After 72 hrs.	After 96 hrs.
None	III	1:10,000,000	Sick.	Sick.	Dead.	
	III	1:10,000,000	"	Dead.		
	III	1:100,000,000	"	"		
	III	1:100,000,000	Dead.			
	I	1:10,000,000	Sick.	Sick.	Dead.	
	I	1:10,000,000	Dead.			
	I	1:100,000,000	Sick.	Dead.		
	I	1:100,000,000	"	"		
Unheated	III	1:10,000,000	"	"		
	III	1:10,000,000	"	Sick.		Well.
	III	1:100,000,000	"	"		"
	III	1:100,000,000	Dead.			
	I	1:10,000,000	Sick.	Sick.		Well.
	I	1:10,000,000	Dead.			
	I	1:100,000,000	Sick.	Sick.		Well.
	I	1:100,000,000	Dead.			
Heated to 130°C. for 3 hrs.	III	1:10,000,000	Sick.	Sick.		Well.
	III	1:10,000,000	"	"	Dead.	
	III	1:100,000,000	"	"		Well.
	III	1:100,000,000	"	"		"
	I	1:10,000,000	"	"		"
	I	1:10,000,000	"	"		"
	I	1:100,000,000	"	"		"
	I	1:100,000,000	"	"		"
Heated to 120°C. for 12 hrs.	III	1:10,000,000	"	"		"
	III	1:10,000,000	"	"		"
	III	1:100,000,000	"	"		"
	III	1:100,000,000	"	"		"
	I	1:10,000,000	"	Dead.		
	I	1:10,000,000	"	Sick.		Well.
	I	1:100,000,000	"	"		"
	I	1:100,000,000	"	"		"

TABLE II.

Protection Test of Mice Treated with Pneumococcus Lipovaccine.

The vaccinating dose was given 38 days before the test. The test consisted of inoculation with the indicated quantity of a 24 hour bouillon culture of Type I pneumococcus intraperitoneally. The total volume injected was 0.5 cc.

Vaccine.	Dilution of culture.	Result.	
		After 24 hrs.	After 48 hrs.
None	1:1,000,000	Sick.	Dead.
	1:1,000,000	"	"
	1:1,000,000	Dead.	
	1:1,000,000	Sick.	Dead.
	1:1,000,000	"	"
	1:1,000,000	Dead.	
	1:1,000,000	Sick.	Dead.
	1:1,000,000	Dead.	
Unheated	1:1,000,000	Well.	Well.
	1:1,000,000	"	"
	1:1,000,000	"	"
	1:1,000,000	"	"
	1:1,000,000	Sick.	"
	1:1,000,000	"	"
	1:1,000,000	"	Dead.
	1:1,000,000	"	Well.
	1:1,000,000	"	"
Heated to 130°C. for 3 hrs.	1:1,000,000	"	"
	1:1,000,000	"	"
	1:1,000,000	"	"
	1:1,000,000	"	"
	1:1,000,000	"	"
	1:1,000,000	"	Dead.

after. As a control the results were compared with those obtained by the administration of three doses of typhoid vaccine in saline suspension intraperitoneally at 5 day intervals. The results are presented in Table III.

The experiment shows that at least for these particular preparations the lipovaccine is less efficient in single doses than is the saline preparation in three doses. The antigenic qualities of the lipovaccine (typhoid) are almost destroyed by heating to 130°C. for 3 hours.

TABLE III.

Agglutinin Production in Rabbits Treated with Typhoid Vaccines.

Rabbit No.	Interval since last dose.	Vaccine.	Serum dilution.								Remarks.
			1:20	1:40	1:100	1:150	1:200	1:500	1:1,000	1:2,000	
	days										
1	16	Saline.	++++	++++	++++	++++	++++	++++	+++	++	27 days after first dose.
2	16	"	++++	++++	++++	++++	++++	++++	+++	+	
3	16	"	++++	++++	++++	++++	++++	+++	++	0	
4	16	"	++++	++++	++++	++++	+++	++	+	0	
5	17	Lipovaccine I	++++	++++	++	0					Rabbits tested 12 days later show no increase in agglutinins.
6	17	" I	++++	++++	++++	++++	++	0			
7	17	" I	++++	++++	++++	++++	+++	0			
8	17	" I	++++	++++	++	0					
9	17	" II	+++	++	0						
10	17	" II	0	0	0	0					
11	17	" II	0	0	0	0					
12	17	" II	++++	+++	++	0					
		Lipovaccine heated to 130°C. for 3 hrs.									
13	16	Lipovaccine I	++	0	0						
14	16	" I	+++	+	0						
15	16	" I	+++	++	0						
16	16	" I	0	0	0						
17	16	" II	+++	++	0						
18	16	" II	0	0	0						
19	16	" II	++++	+++	0						
20	16	" II	0	0	0						

SUMMARY.

Pneumococcus lipovaccine confers a definite protection against pneumococcus infection in mice. The protective quality is not destroyed, and apparently is not greatly diminished, by heating to 130°C. for 3 hours or 120°C. for 12 hours.

Typhoid lipovaccine gives rise to the formation of agglutinins in rabbits but to a lesser degree than saline suspensions. The antigenic qualities of the typhoid lipovaccine are greatly injured by heating to 130°C. for 3 hours.