THE STERILISATION OF VACCINES; AND THE INFLUENCE OF THE VARIOUS METHODS EMPLOYED ON THEIR ANTIGENIC PROPERTIES.

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(With one Chart.)

A VACCINE may, in general, be defined as a standardised suspension of killed bacteria. It is true that in a few instances living organisms are employed as vaccines as in the case of the Haffkine prophylactic for Cholera and the sensitised vaccines of Besredka. Notwithstanding these few exceptions it is suspensions of killed bacteria which are in most general use therapeutically as vaccines. According to Wright's method the bacteria employed in the preparation of a vaccine are killed by heat, the temperature actually employed ranging from 53° C. to 65° C. for one half to two hours. This method of sterilising vaccines has been largely employed, but has recently been subjected to much criticism. It has been stated that employment of heat in the sterilisation of vaccines has impaired the value of the vaccine, the heat coagulating albumin, increasing the toxicity and diminishing the immunising properties of the vaccine.

For these reasons other methods have been introduced, especially the addition of antiseptics. Thus Castellani renders his mixed vaccine sterile by the addition of carbolic acid, Dreyer and Ainley Walker employ formalin in the preparation of typhoid vaccine, whilst Nicolle and Blaizot sterilise their gonorrhoeal vaccine "Dmegon" by the addition of sodium fluoride.

In this way it is claimed that vaccines are deprived of all toxic effects and may be administered without provoking any reaction either local or general. It would seem, however, that these effects are claimed chiefly on clinical grounds. By determining the opsonic index in a

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series of rabbits inoculated with vaccines sterilised in various ways their antigenic value can be accurately determined and compared.

The organism selected for these experiments was a freshly isolated strain of *M. pyogenes* var. *aureus* (*Staphylococcus pyogenes aureus*). The vaccine was made from 48 hour agar slope cultures at 37° C. emulsified in 4 c.c. of sterile physiological saline solution and were sterilised by heating to different degrees, by the addition of selected antiseptics, and by the application of ultra-violet rays. A single dose of 100 million organisms was given in each case. The rabbits were all large healthy stock animals and blood was obtained in a Wright's Capsule from an ear vein. In every case, the blood used as the control was taken at the same time as the samples from the inoculated animals, and the specimens for counting the number of bacteria ingested by the polymorpho-nuclear leucocytes were made in the usual manner within a short time after the animals were bled. Human leucocytes were employed and the counts were made on 100 cells in each case. All the inoculations of the vaccines were made into the subcutaneous tissues of the back.

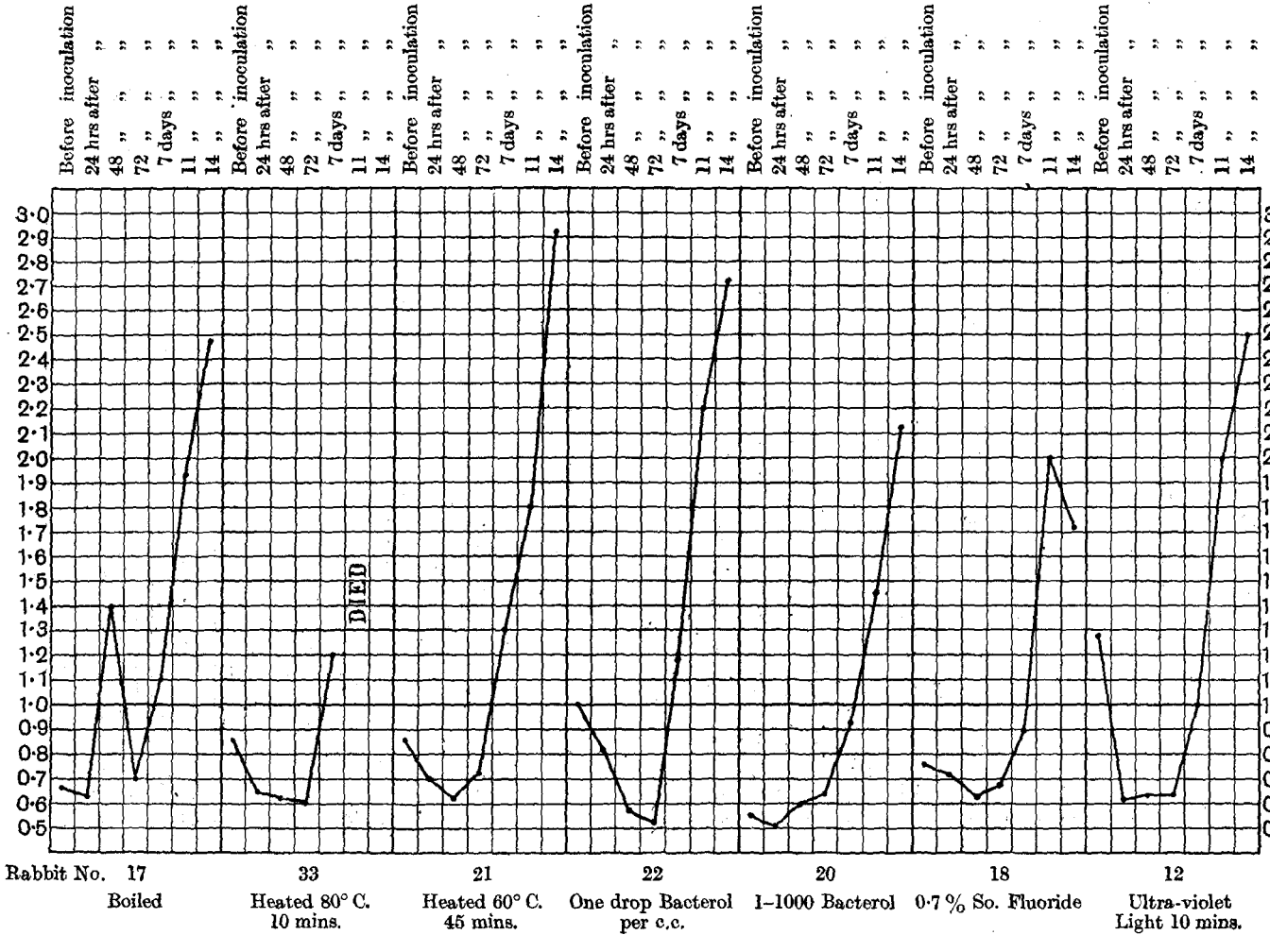
The results obtained are set forth in tabular form in Table I and graphically in Chart I.

TABLE I.

The opsonic indices obtained by the injection of 100 *million staphylococci killed in various ways.*

Rabbit No.	Preparation of Vaccine	Before inoculation	24 hours after inoculation	48 hours after inoculation	72 hours after inoculation	7 days after inoculation	11 days after inoculation	14 days after inoculation
17	Boiled	0·67	0·62	1·40	0·70	1·10	1·93	2·49
33	Heated 80° C. 10 minutes	0·86	0·65	0·62	0·60	1·20	Died	
21	Heated 60° C. 45 minutes	0·86	0·70	0·62	0·73	1·30	1·80	2·92
22	One drop Bacterol per c.c.	1·00	0·82	0·58	0·52	1·19	2·20	2·71
20	Bacterol 1–1000	0·56	0·51	0·60	0·64	0·92	1·45	2·13
18	0·7 per cent. Sodium Flouride	0·76	0·72	0·63	0·68	0·90	2·00	1·71
12	Ultra-violet Light	1·28	0·61	0·65	0·65	1·00	2·00	2·50

CHART I. *Showing the opsonic indices in rabbits injected with a vaccine containing 100 millions of staphylococci killed in various ways, as stated.*



A. *Effects of Heat.*

The organism employed was a somewhat resistant strain of *Staphylococcus pyogenes aureus* to heat, as heating to 60° C. needed to be prolonged to 45 minutes to ensure sterilisation. The vaccine caused a negative phase lasting three days, the opsonic index falling from 0·86 to 0·62 and then steadily rose until it reached 2·92 in fourteen days. Thus a pronounced negative phase was followed by a well-marked positive phase. A very similar course was followed where the organisms were killed by heating to 80° C. for ten minutes. At the end of a week, however, the rabbit had a litter of young and some puerperal complication which cut short the determinations.

Where the organisms were killed by boiling there was only a slight negative phase (during which an anomalous temporary rise of the opsonic index occurred) which was followed by a marked rise to 2·49.

Hence the toxicity of staphylococcus vaccines, as determined by a negative phase in the opsonic index, is not increased by raising the temperature, nor does such a rise of temperature exercise any pronounced diminution of immunising power, as determined by the rise in the opsonic index.

B. *Effects of Antiseptics.*

The addition of three drops of pure Bacterol to the three c.c. of emulsion of staphylococci and subsequent shaking in the shaking machine for half an hour ensured the sterility of the vaccine. This is a simple and rapid method of sterilising vaccines. Bacterol is one of the newer disinfectants containing iodine and formalin, which, while possessing a selective action on spores, is possessed of little toxicity so that several c.c. of 3 per cent. Bacterol can be injected intravenously into rabbits without producing toxic effects.

The vaccine so prepared caused a negative phase for three days, the index falling from 1·0 to 0·52, rising afterwards to reach 2·72 on the fourteenth day. Using a 1–1000 solution of Bacterol, three to four days' exposure was necessary to obtain sterility. In this case the index fell in 24 hours from 0·56 to 0·51 and thereafter steadily rose to 2·4 on the fourteenth day. By the use of Sodium Fluoride in 0·7 per cent. solution, as employed by Nicolle and Blaizot, the index fell in three

days from 0·78 to 0·62, then rose to 2·0 on the eleventh day and fell to 1·71 on the fourteenth day.

Hence the substitution of antiseptics for heat does not definitely decrease the toxicity of the vaccine, nor does it increase the immunising power. On the contrary, it does not reach so high a point as in the heated vaccines and in the case of Sodium Fluoride the positive phase is sooner over.

C. *Effects of Ultra-violet Rays.*

The source of the ultra-violet rays was a quartz mercury vapour lamp using 3½ ampères, and thin layers of emulsions of organisms were exposed to the rays in a Petri dish with the lid removed, the lamp being placed at a distance of six inches. It was found that the thick suspension of organisms obtained by suspending an agar slope culture in three c.c. of saline solution was not nearly sterilised when exposed in quantities of one c.c. in a Petri dish of three inches diameter. The emulsion of living organisms was standardised by counting according to Wright's method and was diluted down so as to contain 1000 million staphylococci per c.c. On exposure of one c.c. of this diluted suspension it was found that an exposure of five minutes usually sufficed to ensure complete sterilisation. Occasionally, however, a few odd colonies developed on the control cultures and exposure for a further period of five minutes ensured sterilisation. It would thus appear that the ultra-violet rays possess an extremely limited power of penetration of any opaque fluid, and this fact militates against their use in the sterilisation of vaccines.

The vaccine sterilised by exposure to the ultra-violet rays for two periods of five minutes each was used for injection, with the result that the opsonic index showed a very marked fall from 1·28 to 0·61 in 24 hours, and then steadily rose, reaching 2·50 on the fourteenth day.

Hence, in addition to the uncertainty in ensuring sterilisation, ultra-violet rays do not diminish the negative phase or toxicity of vaccines, nor do they increase their positive phase or immunising power.

Conclusions.

Taking the opsonic index as a measure of the antigenic powers of a vaccine, its toxicity corresponding with the negative phase, and its immunising powers with the positive phase, it was shown with regard to staphylococcal vaccines that:

(1) Unheated possess no advantages over heated staphylococcal vaccines.

(2) It is unnecessary to limit the amount of heat applied to the minimum that will ensure sterilisation.

(3) Even so high a temperature as boiling does not destroy the antigenic properties of staphylococcal vaccines.

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