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The effect of some antiseptic reagents on the bacterial flora of smallpox lymph

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INTRODUCTION

In previous work (Cardone, 1953, 1956), data were published describing the development of a dehydrated smallpox vaccine made in calf globulin that was more durable than glycerinated vaccines when stored with or without refrigeration. The data included experimental evidence demonstrating that when certain concentrations of phenol and ether were added to lymph vaccine preparations which were ordinarily highly contaminated, the bacterial flora was rapidly diminished (Cardone, 1953).

The lymph used for these experiments, however, was from calves whose inoculated site had been sprayed twice daily with varying concentrations of Roccal* during the viral incubation period. Mention was made that the effects of this treatment might be a bactericidal additive to the action of the phenol and ether which were usually added to the vaccine preparations.

The data that follow derive from further investigations of this factor.

MATERIALS AND METHODS

Materials

Vaccine preparations

These were 20% lymph emulsions ground in calf globulin as described before (Cardone, 1953).

(*a*) Ten preparations, each made to contain 0·5% phenol and 0·8% ether and prepared with a lymph pool derived from calves treated with Roccal during viral incubation period.

(*b*) Ten preparations, each made to contain 0·5% phenol and 0·8% ether and prepared with a lymph pool derived from calves not Roccal-treated during viral incubation period.

(*c*) Ten preparations, each made to contain 0·2% Roccal, 0·5% phenol and 0·8% ether and prepared with a lymph pool derived from calves not Roccal-treated during viral incubation period.

* Alkyl dimethylbenzyl ammonium chloride, marketed by The Winthrop Chemical Co., Windsor, Ont., Canada.

Non-viral 'vaccines' for toxicity tests

The skin of normal rabbits was triturated to a fine pulp to simulate smallpox calf lymph. Three vaccines A, B, and C, were prepared with it as usual (Cardone 1953) and each contained antiseptic reagents as follows:

A vaccine, 0·2 % Roccal, 0·5 % phenol, 0·8 % ether;
B vaccine, 0·2 % Cresol Compound N.F., 0·5 % phenol, 0·8 % ether;
C vaccine, 0·5 % phenol, 0·8 % ether.

Methods

Lyophilization

All the vaccines produced, including the non-viral 'vaccines' for toxicity tests, were lyophilized in small ampoules and sealed in vacuo the morning after preparation as described before (Cardone, 1953).

Bacterial counts

Immediately following lyophilization of the smallpox vaccines described under Materials, (*a*)–(*c*), one ampoule from each lot was opened, reconstituted to original volume with sterile distilled water and then diluted 1/25. One ml. of the dilution was mixed thoroughly with 15 ml. of sterile beef heart agar at 43° C., poured in a sterile Petri dish and allowed to solidify. After incubation for 48 hr. at 37° C., followed by 48 hr. at 20° C., the number of colonies on the plate was counted and multiplied by the dilution factor. The resulting figure represented the number of viable organisms per ml. present in the vaccine.

Toxicity tests: rabbit

The non-viral 'vaccines' described under Materials were reconstituted to original volume with sterile distilled water and tested for toxicity as follows:

(*a*) *Rabbit test, patch method.* 1 ml. each of 'vaccines' A, B and C was dispensed to a fourfold, 1 in. square of sterile gauze. The three patches were cellophane-taped in intimate contact with the depilated skin of a rabbit and removed after 6 hr. The test sites were examined for irritation effect after removal of the patches (6 hr.) and again after 24 , 48 and 72 hr.

(*b*) *Rabbit test, scratch method.* 0·2 ml. separately of A, B and C 'vaccines' was scratched into the depilated skin of a rabbit each in an area 2·5 cm. square. The volume of emulsion was spread over the prescribed site and parallel scratches, about 1 mm. apart, were crisscrossed over the area with a sterile needle. Examinations for irritation effect were done as described above.

Toxicity test: human

Six lots of lyophilized smallpox vaccine, prepared as previously described and containing 0·2 % Roccal, 0·5 % phenol and 0·8 % ether, were pooled and reconstituted to original volume with 50 % glycerine solution. Dispensed in one-dose capillary tubes, they were tested for acceptability of viral potency and bacterial count and were sent to clinics to be used for human vaccinations like any other regularly prepared preparation sent for routine testing.

RESULTS AND DISCUSSION

Data of Table 1, upper half, indicate that smallpox vaccines produced with lymph from Roccal-treated animals and containing 0·5 % phenol and 0·8 % ether show rapid diminution of the bacterial flora. The bactericidal activity occurs, usually, within 24 hr. of vaccine preparation and the bacterial flora reduction is such as to be acceptable to the requirements of the National Institutes of Health

Table 1

Adequate bacterial flora diminution occurs when 0·5 % phenol and 0·8 % ether are added to vaccines prepared with highly contaminated lymph from 'Roccal-treated' calves: the reverse is true when similar vaccines are prepared with lymph from calves not treated with 'Roccal'.

Prep. no.	Bacterial count/ml. of lymph used	Bacterial count/ml. of resulting vaccine preparations containing 0·5 % phenol and 0·8 % ether
	Lymph derived from calves treated with 'Roccal' during viral incubation period	
13	200,000	260
18	160,000	300
19	180,000	320
20	250,000	225
22	155,000	210
25	180,000	275
29	190,000	325
32	175,000	150
34	175,000	160
36	195,000	210
	Lymph derived from calves *not* treated with 'Roccal'	
26, 27, 35, 42, 47, 48, 49, 107, 108, 109	Uncountable	Uncountable

(U.S. Department of Health, 1951) and as was reported in previous publications (Cardone, 1953, 1956). Data of Table 1, lower half, on the contrary, show that when the same concentrations of phenol and ether are added to vaccines prepared with lymph from animals not Roccal-treated during the viral incubation period the bacterial flora diminution is not adequate.

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These results suggest that lymph from Roccal-treated animals might contain adsorbed quantities of the reagent (Cardone & Mazzarella, 1938) which act as an additive to the bactericidal activity of the phenol and ether.

Table 2

Successful diminution of bacterial flora is obtained when 0·2 % 'Roccal', 0·5 % phenol and 0·8 % ether are added to vaccines prepared with lymph from calves not treated with 'Roccal'.

Prep. no.	Bacterial count/ml. of lymph used	Bacterial count/ml. of vaccine preparations containing 0·2 % 'Roccal', 0·5 % phenol and 0·8 % ether
105	Uncountable	310
110		436
111		140
112		264
113		180
114		120
115		210
116		190
118		290
120		75

Proof of this premise might have been determined readily by direct chemical assay of Roccal in lymph; however, it was found impossible to do so since protein digestion procedures necessary for such work destroy the reagent. A bacteriological approach, then, had to be adopted. It was done by adding to each of a series of vaccines prepared with lymph from animals not Roccal-treated increasing concentrations of Roccal together with the constant concentrations of 0·5 % phenol and 0·8 % ether. Bacterial counts revealed that the vaccine containing 0·2 % Roccal showed a diminution of bacterial flora comparable to that obtained when 0·5 % phenol and 0·8 % ether are added to vaccines prepared with lymph from Roccal-treated animals.

To substantiate this finding some one hundred vaccines were prepared with lymph from animals not Roccal-treated and containing 0·2 % Roccal, 0·5 % phenol and 0·8 % ether. Data of Table 2 not only show the adequate bacterial flora diminution in ten preparations representative of the series, but give evidence that lymph from Roccal-treated animals contains adsorbed, or has the additive bactericidal effects of the use of, at least 0·2 % Roccal when in final vaccine dilution of 20 % emulsion.

The Roccal effect, it has been observed, is variable from animal to animal due to variations in scarification techniques, skin conditions, etc., therefore it has been found preferable to add the recommended concentrations of Roccal, phenol and ether to vaccines at the time of their preparation with lymph from animals not Roccal-treated.

Roccal has been reported to cause no dermatitis in man (Walter, 1938). The

possibility that the combination of Roccal, phenol and ether might cause dermal toxic effects was, however, investigated. Data of Table 3 show that vaccine preparations containing the combination of reagents cause negligible irritation effect in the rabbit and none in sixty-three vaccinated humans.

Table 3

Non-viral 'vaccines' containing 0·2% 'Roccal', 0·5% phenol and 0·8% ether reconstituted from the dehydrated state cause negligible irritation to the skin of the rabbit. In human vaccinations, smallpox vaccines containing the same reagents and reconstituted from the dehydrated state cause no reaction unusual with 'takes'.

Type of test	Number of hours from test beginning and degree of irritation at test sites*. 6	24	48	72
I. Rabbit test				
1. Patch method				
A. 'vaccine' containing: 0·2% 'Roccal', 0·5% phenol, 0·8% ether	0	0	±	0
B. (Control) 'vaccine' containing: 0·2% cresol compound N.F., 0·5% phenol, 0·8% ether	0	0	±	0
C. (Control) 'vaccine' containing: 0·5% phenol, 0·8% ether	0	0	0	0
2. Scratch Method				
A. 'vaccine', above	0	±	0	0
B. 'vaccine', above	0	±	0	0
C. 'vaccine', above	0	0	0	0

* 0, No reaction; ±, slight reddening of the skin.

II. Human test	Reactions and number of days after vaccination 2	7	14
Sixty-three children were vaccinated with a reconstituted lyophilized vaccine pool containing the reagents as in A above	No reactions	No reactions unusual with smallpox 'takes'	

The use of Roccal in the concentration described did not affect the viral potency of the vaccines when tested in the rabbit over a period well over 2 years (Cardone, 1953).

SUMMARY

Data are presented which amplify previously published work on the additive antiseptic effect of Roccal as follows:

1. Smallpox calf pulp derived from animals whose skin is treated with Roccal during the viral incubation period apparently adsorbs sufficient concentration of the reagent to produce a measurable bactericidal effect.

2. The adsorbed antiseptic induces a significant additive effect well beyond

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that of the action of stated concentrations of phenol and ether alone, as evidenced by the fact that this combination produces rapid diminution of the bacterial flora in highly contaminated calf lymph.

3. Vaccines containing the combination of antiseptics described caused no dermatitis or other untoward reactions in rabbits and humans and their viral potency was not affected.

4. The use of the antiseptic reagents makes possible the rapid preparation of smallpox vaccines for human use. Acceptable bacterial flora levels are obtained usually within 24 hr. of preparation.

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