RESEARCH PAPERS

THE SURVIVAL OF STREPTOCOCCI AND TUBERCLE BACILLI IN OILS

WITH SPECIAL REFERENCE TO THOSE OF PHARMACEUTICAL INTEREST

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INTRODUCTION

THE administration of drugs by injection has resulted in many bacterial infections, some of which have been fatal[1,2,3]. Much attention has therefore been given to all aspects of the subject and many papers have appeared upon:—(a) the sterilisation of the syringe and needle, for example, by Bigger *et al.*[4] and M.R.C. War Memorandum No. 15[5]; (b) the disinfection of the skin around the site of injection, e.g., by Archer[6] and Gardner[7]; (c) the asepsis of the injection and its container.

With regard to (c), studies have been made of the aseptic precautions to be taken during the process of manufacture and distribution into the final containers, and in respect of the laboratories, equipment and staff concerned (Holt[8] and Coulthard[9]). Where heat treatment adequate to ensure sterility is not possible, non-toxic and efficient bactericides have been found to supplement the action of relatively mild degrees of heat (Coulthard[10,11], Davis[12], Berry *et al.*[13] and others[14]). Heat in any form is sometimes inadmissible. Much attention has, therefore, been given to filtration processes and many papers have appeared, Sykes and Royce[15] found, in their preliminary experiments, that relatively unprotected cells of *Bacterium prodigiosum* became non-viable in the space of a few hours when suspended in oils commonly used as vehicles for injections.

Many of the results of this work have been reflected in official publications, such as the British Pharmacopœia, which have concerned themselves also with tests to ensure sterility and with other aspects of injections, including the precautions to be taken to prevent the growth of organisms accidentally introduced if the injection is nutrient and dispensed in multiple dose containers. It is not generally regarded as necessary, even with aqueous solutions, to add a bactericide to deal with accidentally introduced bacterial contaminants which may be surviving but not multiplying.

The chief ways by which injections become infected would appear to be (a) the transference into the container of bacteria on the external

surface of the cap of the vial, on the needle, in the syringe, in the air, or from the hands of the operator, or (b) the transference of infective material from one patient to a multidose container, with subsequent infection of other patients (Hughes[16], Evans and Spooner[17]). Bigger *et al.*[18] showed that (b) could occur with toxoid-antitoxin floccules but in the presence of 0·5 per cent. of phenol even heavy inocula of tubercle bacilli failed to survive after 7 days.

It is sometimes asked if a bacteriostatic should be added to multiple dose containers of oily injections. It is obviously unlikely that pathogenic bacteria would multiply in oil, but flooding with oil has been found satisfactory as a method of preserving cultures and it seemed desirable that the survival of bacteria in oily injections should be studied. They appear to have been somewhat neglected, perhaps because sterility tests, devised for aqueous solutions, are less satisfactory with oils, and because of a common assumption that bacteria in oils rapidly die out.

Partly as a result of a request by the Sub-Committee on Sterile Materials of the British Pharmacopœia Commission, it was decided to study some aspects of the problems involved and particularly the survival of pathogenic organisms in oils and the effect of added bactericides upon this survival. A survey was first made of the literature of the survival of bacteria in oils and in other environments. A number of papers have appeared dealing with the action of oils on the tubercle bacillus and the survival of bacteria in "cutting" and other oils. On the former point, Crimm and Martos[19] give a brief résumé of earlier papers which mention a lytic action of mineral and vegetable oils, and an inhibitive effect of certain oils in culture media accompanied by alteration of staining properties of the organisms. They then describe their own experiments, which in some cases fail to confirm the results of earlier workers, and conclude that peanut oil, cod-liver oil and gomenol incorporated in small amounts in culture media are inhibitory for tubercle bacilli. They did not find that any of these three oils, even after prolonged contact, altered the virulence or acid-fastness of strain H37 or that complete lysis occurred, and concluded that the inhibitory effect of the oils was due to a physical rather than a chemical action.

Corper and Cohn[20], in their studies on the effect of paraffin hydrocarbons on tuberculo-allergy and tuberculo-immunity, found that storage of human tubercle bacilli at incubator temperature (37°C.) in suspension in mineral oil resulted in rapid loss of viability of the bacilli (within 2 to 4 days). A retardation of this effect occurred at refrigerator temperature. Fraser[21] studied the survival of *Bacterium coli* and staphylococci in a cutting oil (a medium heavy mineral oil), in the presence and absence of a phenolic antiseptic, and found that when the antiseptic was present these bacteria were destroyed in 24 hours. He does not seem to have determined the survival period in the absence of antiseptic. Leusden and Derlich[22], using suspensions of bacteria added direct to cod-liver oil or mixed in liquid paraffin and then added to the oil, found that staphylococci, streptococci, *Bacterium coli* and *Bacterium typhosum* were killed

after 30 minutes to 4 hours. The survival time depended on the method of making the suspension and on the cod-liver oil used. Löhr[23] found that staphylococci, streptococci and *Bacterium coli* lost their vitality in cod-liver oil in 3 hours, but survived in liquid paraffin for more than 14 days. Lichtenstein[24], who gives a survey of the work on the bactericidal power of oils, found that *Bacterium coli* and staphylococci in the absence of a nutrient medium survived in cod-liver oil for 21 hours, in linseed oil for about 1 hour, in olive oil 8 to 9 days and in liquid paraffin 35 days, the longest period tested.

There are numerous references to the survival of bacteria in a variety of environments. The widespread use of freeze-drying for the preservation of cultures is evidence of the fact that bacteria are able to survive in a resting condition for many years. Frobisher *et al.*[25], for example, examined vacuum desiccated cultures of various strains of tubercle bacilli, which had been in the refrigerator for 17 years, and found all viable and most of them virulent. Other workers[26,27] have shown that streptococci and meningococci resisted freeze-drying. We have ourselves kept freeze-dried cultures of *Streptococcus hæmolyticus* for several years without loss of virulence for mice. McCulloch[28] states that " protected from oxygen and moisture and held at low temperatures microorganisms are able to live for long periods of time, although such populations are constantly decreasing." Buchbinder and Phelps[29] and Buchbinder, Solowey and Solotorovsky[30] using their experimental room found that in the dark, the estimated time for 50 per cent. survival for 3 strains of streptococci varied from 1 to 13 days and most of the cells of one strain of β-hæmolytic streptococci exposed for about 10 days did not alter their virulence for mice. Corper and Cohn[31] found that human and bovine tubercle bacilli could survive without loss of virulence in glycerol broth incubated at 37°C. for 12 to 13 years. They noted that only those cultures survived in which the medium was at a *p*H between 6·1 and 7·6. Potter[32] stated that cultured avian strains of tubercle bacilli could live up to 2 years completely deprived of air and water. Smith[33] found that human tubercle bacilli could survive on cover slips for over 6 months in the refrigerator, for 2 or 3 months in darkness at room temperature but in light usually for less than 4 days. He found that apparent survival of tubercle bacilli was rather longer when tested by guinea-pig inoculation than by culture methods. The same worker[34] reported that tubercle bacilli survived in books for periods ranging from 16 to 112 days depending on the type of paper used. In all this work, the survival time was longer in the winter with lower temperature and humidity and also when the initial population was larger.

The experiments we report in this paper with tubercle bacilli and streptococci were designed to parallel the circumstances when an infection is introduced from a patient into a multidose container. In view of the difficulties of carrying out sterility tests in oils or of enumerating any bacteria they may contain, it was decided to use virulent bacteria and to test for survival by animal inoculation. The selection of animals to test for survival presented some difficulty. We particularly wished to

include the oily injection of penicillin in our experiments, but guinea-pigs, the animals of choice for detecting tubercle bacilli, are unusually susceptible to penicillin. This has been reported by several workers (e.g., Cornia, Lewis and Hopper[35]). Rivière, Thely and Gautron[36] reported that experimental tuberculosis in guinea-pigs was increased in severity by penicillin treatment, and Hauduroy and Rossett[37] concluded that penicillin had no action on tuberculosis in this animal and that the effect was due to toxicity.

Omitting the penicillin and testing the base only appeared to be the best way out of this difficulty. It would appear unlikely that penicillin is highly lethal for tubercle bacilli and it has indeed been reported that, in small amounts, this antibiotic increased the growth of tubercle bacilli (Ungar and Muggleton[38]), although this enhancement was subsequently found to be due to the improved oxygen supply resulting from frequent opening of the test cultures for penicillin assay (Iland and Baines[39]). Iland[40] found that recently isolated strains were inhibited by penicillin but this was not confirmed by Kirby and Dubos[41], unless Tween 80 was present in the medium. More recently, Iland and Baines[39] have shown that a virulent laboratory strain of tubercle bacilli produced a penicillin-destroying substance with enzyme-like properties similar to those of penicillinase from other organisms.

After consideration of this evidence it was decided to use guinea-pigs for our work with tubercle bacilli, and when oily injections of penicillin were under test to inject the base omitting the penicillin. The tests for surviving streptococci were made in mice. It is well known that many common bactericides and bacteriostatic agents are markedly reduced in efficiency when dissolved in oil and it seemed of interest to include in our tests some oils containing phenol or other bactericides.

Experimental

(A). *Tests using hæmolytic streptococci.* 5 ml. samples of each vehicle were distributed into plain 1 oz. bottles of the type used for penicillin. The streptococci in blood were then added (0·1 ml. in the first test and 1 drop from a Pasteur pipette subsequently) and the bottle was rubber plugged, shaken vigorously in the hand, and allowed to stand in the dark at room temperature or in the refrigerator. In one test the period extended to 37 weeks in the refrigerator. One container was used for each time interval. After vigorous shaking it was opened, the contents were withdrawn by syringe and 0·25 ml. was injected intraperitoneally into each of the indicated number of 20 g. mice. These mice were then kept for 4 days and the deaths noted. In many cases the cause of death was confirmed by spleen culture.

In the first test the bottles were sown with a mixture of equal parts of normal horse-blood and a 24-hour serum broth culture of *Streptococcus hæmolyticus* (Richards). In subsequent tests, infected mouse blood was used. This was obtained by injecting mice intraperitoneally with a lethal dose of streptococci; as soon as approximately 25 per cent. had died, the

remainder of the group were killed with chloroform and immediately bled from the heart, potassium oxalate being used as an anticoagulant. The number of contained streptococci was estimated by plate-count. Whilst this technique appeared to conform closely to the conditions of infection considered by Bigger *et al.*[18], Evans and Spooner[17], and Hughes[16], it introduced considerable difficulties in standardising the numbers, resistance and virulence of the streptococci. The blood tended to separate out on storage as a granular deposit. It was stirred up with a platinum loop before shaking the bottle.

Initially, ethyl oleate was included in the series. This, however, had to be omitted owing to its surprising toxicity when given intraperitoneally. It was found that as little ethyl oleate (B.P.) as 0·05 ml. caused death in 2 out of 4 mice within 2 days, while 0·3 ml. killed 5 out of 5 mice within 4 hours. This toxicity is not displayed when it is given intramuscularly or subcutaneously.

(B). *Tests using tubercle bacilli.* 2 ml. samples of each vehicle were distributed into amber 1 oz. bottles fitted with rubber bungs. These were infected as indicated below and after very thorough shaking were allowed to stand in the dark at room temperature or in the refrigerator. Immediately before sampling each bottle was thoroughly shaken. The tubercle bacilli used were of human type (H418 or H37Rv) and in the first three tests the numbers were kept low so that they might represent the infection likely to be introduced by an infected syringe. In test 2 the number of bacilli was not determined owing to the difficulties in making accurate counts of tubercle bacilli. In the fourth test, which included samples to which preservatives had been added, the numbers of bacteria were increased. One container was used for each sampling; it was placed in the incubator at 37°C. for 30 minutes, shaken thoroughly and then the contents removed as completely as possible by syringe and distributed equally by intraperitoneal injection into 3 guinea-pigs.

The presence of living virulent tubercle bacilli was detected by:— (a) Tuberculin tests on the living guinea-pigs, using 0·2 ml. of a 1/100 or 1/50 dilution of old tuberculin. (b) Macroscopic examination of spleen, liver, lungs, and glands of the killed animals. (c) Culture of the spleen on Löwenstein medium.

Each bottle received:—

In Test 1, about 1000 moist bacilli (H418) from modified Dubos[42] medium.

In Test 2, 0·02 ml. of a suspension in blood of an infected spleen. The spleen was taken from a guinea-pig 3 weeks after it had been infected with 0·5 ml. of a 1/100 dilution of a culture of H418 in modified Dubos[42] medium.

In Test 3, about 1000 dry bacilli (H37Rv) dried on a kaolin and French chalk mixture at 37°C. for 24 hours.

In Test 4, about 16 million bacilli (H418) added as equal parts of a modified Dubos[42] culture and blood.

RESULTS

Details of our experiments are given in Tables I to IV.

Streptococci. Our results showed that, at room temperature, streptococci in blood may remain virulent in liquid paraffin for at least 8 weeks and in arachis oil for at least 4 weeks. The addition of phenol 0·5 per cent. to the arachis oil considerably reduced the period over which virulent organisms could be detected. Certain other germicides produced a similar effect. At refrigerator temperature virulent streptococci may

TABLE I

THE SURVIVAL OF HÆMOLYTIC STREPTOCOCCI IN OIL IN CONTAINERS STORED AT ROOM TEMPERATURE

Test Substance	Test	Number of streptococci per dose of 0·25 ml.	Deaths of mice (from groups of 5) inoculated after the vehicle had been stored for (weeks or for fractions of a week)								Survival of virulent streptococci (in weeks)	
			0	1/7	3/7	1	2	3	4	8	Present at—	Not detected at—
Liquid paraffin alone ...	1	6,000	5*	5*	3	5*	–	–	5	5	8	–
Arachis oil alone			5*	4*	5*	5*	–	–	0	0	1	4
Arachis oil + 0·5 per cent. of phenol			5*	3*	4	4*	–	–	0	0	1	4
Arachis oil + 4·5 per cent. of beeswax			5*	4*	0	0	–	–	0	1*	8	–
Liquid paraffin alone ...	2	140	5	–	5*	5	5	–	5	–	4	–
Arachis oil alone			5	–	5*	3	5	–	1	–	4	–
Arachis oil + 0·5 per cent. of phenol			5	–	2*	0	0	–	0	–	3/7	1
Arachis oil + 4·5 per cent. of beeswax			5	–	2*	1	0	–	0	–	1	2
Arachis oil alone	3	54,000	5*	5	–	0	–	–	0	0	1/7	1
Arachis oil + 1·0 per cent. of Phenol			5*	5	–	0	–	–	0	0	1/7	1
Arachis oil + 0·5 per cent. of cresol			5*	5	–	1	–	–	0	0	1	4
Arachis oil + 0·2 per cent. of hexyl-resorcinol ...			5*	5	–	0	–	–	0	0	1/7	1
Arachis oil + 1·0 per cent. of camphor			5	5	–	2	–	–	0	0	1	4
Arachis oil + 0·1 per cent. of methyl *p*-hydroxybenzoate			5*	0	–	1	–	–	0	0	1	4
Arachis oil + 0·5 per cent. of chlorocresol			5*	5	–	0	–	–	0	0	1/7	1
Arachis oil alone	4	1,125	4†	0‡	0	–	1*	–	–	–	2	–
Arachis oil + 1·0 per cent. of phenol			5†	0‡	1	–	0	–	–	–	3/7	2
Arachis oil + 0·5 per cent. of cresol			5†	0‡	0	–	0	–	–	–	0	1/7
Arachis oil + 0·2 per cent. of hexyl-resorcinol ...			3†	0‡	1*	–	0	–	–	–	3/7	2
Arachis oil + 0·5 per cent. of chlorocresol			5†	1‡	1	–	0	–	–	–	3/7	2
Arachis oil + 1·0 per cent. of camphor			5†	5‡	0	–	5*	–	–	–	2	–
Arachis oil + 0·1 per cent. of propyl *p*-hydroxybenzoate			4†	0‡	0	–	1	–	–	–	2	–
Arachis oil only	5	28,000	5	–	5*	4*	0	–	–	0	1	2
Arachis oil + 1·0 per cent. of phenol			5	–	5*	0	0	–	–	1*	8	–
Arachis oil + 0·5 per cent. of cresol			5	–	5*	0	0	–	–	0	3/7	1
Arachis oil + 0·1 per cent. of propyl *p*-hydroxybenzoate			5	–	5*	1	0	–	–	0	1	2

* The presence of hæmolytic streptococci was proved by autopsy and culture on blood agar.
† 3 day record. ‡ 2 day record.

survive for over 37 weeks in arachis oil and at least 4 weeks in liquid paraffin. The addition of 1 per cent. or 0·5 per cent. of phenol or some other bactericides considerably reduced the period over which they could be detected.

Tubercle Bacilli. Our results showed that, at room temperature, small numbers of tubercle bacilli in blood remain viable and virulent in saline solution and arachis oil for 10 weeks but not for 26 weeks. In arachis oil

TABLE II

THE SURVIVAL OF HÆMOLYTIC STREPTOCOCCI IN OIL IN CONTAINERS STORED IN THE REFRIGERATOR

Test Substance	Test	Number of streptococci per dose of 0·25 ml.	Deaths of mice (from groups of 5) inoculated after the vehicle had been stored for (for weeks or for fractions of a week)									Survival of virulent streptococci (in weeks)	
			0	1/7	3/7	1	2	3	4	8	37	Present at—	Not detected at—
Liquid paraffin alone...	1	6,000	5*	5*	5*	5*	–	–	5	0	–	4	8
Arachis oil alone ...			5*	5*	0	5*	–	–	5	4*	–	8	–
Arachis oil + 0·5 per cent. of phenol ...			5*	5*	5*	5*	–	–	2	0	–	4	8
Arachis oil + 4·5 per cent of beeswax ...			5*	5*	5*	3	–	–	1	0	–	4	8
Liquid paraffin	2	140	5	–	5*	5	5	–	1	–	–	4	–
Arachis oil alone ...			5	–	5	5	1	–	0	–	–	2	4
Arachis oil + 1·0 per cent. of phenol ...			5	–	5	2	1	–	0	–	–	2	4
Arachis oil + 4·5 per cent. of beeswax ...			5	–	5*	5	5	–	0	–	–	2	4
Arachis oil alone ...	3	54,000	5*	5	–	5	–	–	5*	2	–	8	–
Arachis oil + 1·0 per cent. of phenol ...			5*	5	–	0	–	–	0	0	–	1/7	1
Arachis oil + 0·5 per cent. of cresol ...			5*	5	–	5	–	–	1	0	–	4	8
Arachis oil + 0·2 per cent. of hexyl-resorcinol			5*	5	–	5	–	–	5*	0	–	4	8
Arachis oil + 0·5 per cent. of chlorocresol			5*	5	–	1	–	–	0	0	–	1	4
Arachis oil + 1·0 per cent. of camphor ...			5*	5	–	5	–	–	5*	0	–	4	8
Arachis oil + 0·1 per cent. of methyl-*p*-hydroxybenzoate ...			5*	5	–	5	–	–	0	0	–	1	4
Arachis oil alone ...	4	1,125	4†	4‡	2*	–	1	–	–	–	2*	37	–
Arachis oil + 1·0 per cent. of phenol ...			5†	0‡	0	–	0	–	–	–	0	0	1/7
Arachis oil + 0·5 per cent. of cresol ...			5†	5‡	0	–	1*	–	–	–	0	2	–
Arachis oil + 0·2 per cent. of hexyl-resorcinol			3†	4‡	3*	–	1*	–	–	–	0	2	–
Arachis oil + 0·5 per cent. of chlorocresol			5†	4‡	1	–	0	–	–	–	0	3/7	2
Arachis oil + 1·0 per cent. of camphor ...			5†	0‡	2*	–	0	–	–	–	0	3/7	2
Arachis oil + 0·1 per cent. of propyl *p*-hydroxybenzoate ...			4†	0‡	0	–	0	–	–	–	0	0	1/7
Arachis oil alone ...	5	28,000	5	–	5*	5*	–	5*	–	0	–	3	8
Arachis oil + 1·0 per cent. of phenol ...			5	–	5*	5*	–	0	–	0	–	1	3
Arachis oil + 0·5 per cent. of cresol ...			5	–	5*	5*	–	4*	–	0	–	3	8
Arachis oil + 0·1 per cent. of propyl *p*-hydroxybenzoate ...			5	–	5*	5*	–	5*	–	0	–	3	8

* The presence of hæmolytic streptococci was proved by autopsy and culture on blood agar.
† 3 day record. ‡ 2 day record.

with aluminium stearate the period of viability was less than 4 weeks. The addition of 0·5 per cent. of phenol reduced the period of viability to less than 2 weeks with saline solution and less than 4 weeks with arachis oil. Propyl *p*-hydroxybenzoate did not show a similar advantage during our time periods. In the preliminary experiment using arachis oil with beeswax the tubercle bacilli remained viable for over 5 weeks. no tests were made over a longer period, and the effect of added bacteri-

TABLE III

THE SURVIVAL OF TUBERCLE BACILLI IN OIL IN CONTAINERS STORED AT ROOM TEMPERATURE

Test Substance	Test	Type of infection and original (approx.) number of organisms per dose per guinea-pig	T.B. positive guinea-pigs (from groups of 3*) after storage of vehicles for— Weeks							Survival of Tubercle Bacilli	
			0	1	2	4	5	10	26	Present at (Weeks)	Not detected at (Weeks)
Saline (Controls) ...	1	Moist T.B.; Strain H418, about 300 organisms	3	3	–	–	2	j–	–	5	–
Arachis oil + 4 per cent. of beeswax...		,, ,, ,, ,,	3	2/2	–	–	2	–	–	5	–
Arachis oil + 2 per cent. of aluminium stearate		,, ,, ,, ,,	3	2	–	–	0	–	–	1	5
Saline (Controls) ...	2	T.B. in blood; Strain H418, No. of organisms unkown ...	3	2	–	–	2/2	–	–	5	–
Arachis oil + 4 per cent. of beeswax...		,, ,, ,, ,,	3	1/1	–	–	1	–	–	5	–
Arachis oil + 2 per cent. of aluminium stearate		,, ,, ,, ,,	1	2/2	–	–	0	–	–	1	5
Saline (Controls) ...	3	Dry T.B.; Strain H37Rv, about 300 organisms	2	1/2	–	–	0	–	–	1	5
Arachis oil + 4 per cent. of beeswax...		,, ,, ,, ,,	1	1	–	–	1	–	–	5	–
Arachis oil + 2 per cent. of aluminium stearate		,, ,, ,, ,,	3	2	–	–	0	–	–	1	5
Saline (Controls) ...	4	T.B. in blood; Strain H418, about 5 million organisms	3	3	–	3	–	3	0	10	26
Arachis oil		,, ,, ,, ,,	3	3	–	3	–	3	0	10	26
Arachis oil + 2 per cent. of aluminium stearate		,, ,, ,, ,,	3	3	–	0	–	0	0	1	4
Saline + 0·5 per cent. of phenol ...		,, ,, ,, ,,	3	1	0	0	–	0	–	1	2
Arachis oil + 2 per cent. of aluminium stearate + 0·5 per cent. of phenol ...		,, ,, ,, ,,	3	2/2	3	0	–	0	–	2	4
Saline solution + 0·1 per cent. of propyl *p*-hydroxybenzoate†		,, ,, ,, ,,	3	2	0	0	–	0	–	1	2
Arachis oil + 0·1 per cent. of propyl-*p*-hydroxybenzoate		,, ,, ,, ,,	3	3	3	2/2	–	3	–	10	–
Arachis oil + 2 per cent. of aluminium stearate + 0·1 per cent. of propyl-*p*-hydroxybenzoate		,, ,, ,, ,,	3	3	3	3	–	3	–	10	–
		,, ,, ,, ,,	3	2	0	0	–	0	–	1	2

* Groups of less than 3 guinea-pigs dying from non-specific causes are shown thus 2/2.
† Saturated solution of propyl *p*-hydroxybenzoate.

cides was not studied. At refrigerator temperature tubercle bacilli showed a general tendency to survive longer than at room temperature, survival in saline solution and arachis oil occurring for at least 26 weeks. The beneficial effect shown by phenol in arachis oil at room temperature was not found over the time period covered by storage in the refrigerator. Tubercle bacilli dried by the method described in the preliminary experiment appeared to be lowered in resistance.

TABLE IV

THE SURVIVAL OF TUBERCLE BACILLI IN OIL IN CONTAINERS STORED AT 2°C.

Test Substance	Test	Type of infection and original (approx.) number of organisms per dose per guinea-pig	T.B. positive guinea-pigs (from groups of 3*) after storage of vehicles for— Weeks							Survival of Tubercle Bacilli	
			0	1	2	4	5	10	26	Present at (Weeks)	Not detected at (Weeks)
Saline (Controls) ...	1	Moist T.B.; Strain H418, about 300 organisms...	3	3	–	–	2	–	–	5	–
Arachis oil + 4 per cent. of beeswax...		,, ,, ,, ,,	3	2	–	–	2	–	–	5	–
Arachis oil + 2 per cent. of aluminium stearate		,, ,, ,, ,,	3	1/2	–	–	0	–	–	1	5
Saline (Controls) ...	2	T.B. in blood; Strain H418, No. of organisms unknown ...	3	2	–	–	3	–	–	5	–
Arachis oil + 4 per cent. of beeswax...		,, ,, ,, ,,	3	1	–	–	2	–	–	5	–
Arachis oil + 2 per cent. of aluminium stearate		,, ,, ,, ,,	1	2	–	–	0	–	–	1	5
Saline (Controls) ...	3	Dry T.B.; Strain H37Rv, about 300 organisms	2	3	–	–	0	–	–	1	5
Arachis oil + 4 per cent. of beeswax...		,, ,, ,, ,,	1	1	–	–	0	–	–	1	5
Arachis oil + 2 per cent. of aluminium stearate		,, ,, ,, ,,	3	1	–	–	0	–	–	1	5
Saline (Controls) ...	4	T.B. in blood; Strain H418, about 5 million organisms	3	3	–	3	–	3	3	26	–
Arachis oil		,, ,, ,, ,,	3	3	–	3	–	3	3	26	–
Arachis oil + 2 per cent. of aluminium stearate		,, ,, ,, ,,	3	3	–	0	–	0	0	1	4
Saline solution + 0·5 per cent. of phenol		,, ,, ,, ,,	3	0	–	0	–	0	–	0	1
Arachis oil + 0·5 per cent. of phenol ...		,, ,, ,, ,,	3	2/2	3	3	–	3	–	10	–
Arachis oil + 2 per cent. of aluminium stearate + 0·5 per cent of phenol ...		,, ,, ,, ,,	3	2/2	3	3	–	0	–	4	10
Saline solution + propyl *p*-hydroxybenzoate†		,, ,, ,, ,,	3	3	3	3	–	3	–	10	–
Arachis oil + 0·1 per cent. of propyl *p*-hydroxybenzoate		,, ,, ,, ,,	3	3	3	3	–	3	–	10	–
Arachis oil + 2 per cent. of aluminium stearate + 0·1 per cent. of propyl *p*-hydroxybenzoate...		,, ,, ,, ,,	3	3	3	1	–	0	–	4	10

* Groups of less than 3 guinea-pigs dying from non-specific causes are shown thus 2/2.
† Saturated solution of propyl *p*-hydroxybenzoate.

FURTHER WORK

The work reported in this paper was planned upon a basis of relatively short survival periods as indicated by some of the papers we quote in the introduction, consequently fresh experiments will be necessary to cover more prolonged periods. These may well not apply for bacteria not protected by blood as is indeed suggested by our tests with dried tubercle bacilli and we now have further tests in hand using dried bacteria. We are also following up the suggestion that aluminium stearate may exert a bactericidal effect.

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