STUDIES ON THE SUSCEPTIBILITY OF *RICKETTSIA BURNETI* TO CHEMICAL DISINFECTANTS, AND ON TECHNIQUES FOR DETECTING SMALL NUMBERS OF VIABLE ORGANISMS

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(With 4 Figures in the Text)

INTRODUCTION

The discovery of the causative agent of Q fever in the milk of dairy cows by Huebner, Jellison, Beck, Parker & Shepard (1948) naturally led to studies on the susceptibility of this organism to heat, such as that used in pasteurization procedures. Both Huebner, Jellison, Beck & Wilcox (1949) and Marmion, MacCallum, Rowlands & Thiel (1951), showed that *Rickettsia burneti* was unexpectedly heat resistant and that the temperatures ordinarily used in pasteurization left little margin for safety. Epidemiological findings also suggest that *R. burneti* can survive long periods in contaminated buildings and pastures, at least in temperate climates.

Although no detailed comparisons have been made, these findings suggest that *R. burneti* is a more hardy organism than the other pathogenic rickettsiae, which loose infectivity more rapidly under similar conditions. We therefore decided to study the susceptibility of *R. burneti* to chemical disinfectants and in particular, to those which are commonly used in the laboratory. For example, formalin is used to destroy infectivity of yolk-sac suspensions before vaccine and antigen production and ether is also used in this process to assist purification. Considerable faith is sometimes placed in such commercial preparations as 'Lysol', 'Dettol' and 'Cetavlon' for dealing with general laboratory contamination.

Additional stimulus for an investigation of chemical agents came from an unexpected quarter. Vaccine lymph is prepared in Britain from sheep at the Lister Institute. As these animals are possible reservoirs of *R. burneti*, it was suggested that vaccine lymph might be contaminated with these organisms. Bacteria in the lymph are reduced in number by treatment with 1·0 % phenol and we were asked by Dr D. McClean, who is in charge of the vaccine lymph production, to find out whether *R. burneti* is also susceptible to this agent.

While this investigation was in progress similar studies were reported by Ransom & Huebner (1951). These workers extended previous research (Huebner *et al.* 1949) on the effect of heat on *R. burneti* and also investigated the effect of formalin and phenol. They found that these two agents, even in concentrations of 1 %, failed to inactivate *R. burneti*, while other rickettsiae were all killed even by 0·1 % formalin or 0·5 % phenol. Nevertheless, *R. burneti* resembled the other

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pathogenic rickettsiae tested in its susceptibility to ether and to ultra-violet light. Ransom & Huebner stress the importance of techniques which will detect a very small residuum of live organisms and by comparing infectivity titrations in fertile hens' eggs, adult guinea-pigs and weanling guinea-pigs, they found that eggs were the most sensitive for detecting small numbers of rickettsiae provided at least two blind sub-passages were carried out. Comparative titrations carried out in this laboratory have amply confirmed this observation. Fertile eggs have the additional advantage of giving, by the survival time of the embryos after inoculation, an approximate estimate of the infectivity of the inoculum. This can be used in quantitative comparisons of different disinfectants.

This paper describes experiments to test the reaction of phenol, formalin, 'Cetavlon', 'Dettol', 'Lysol' and ether on the viability of *R. burneti*. The effect of temperature on the action of these agents was also investigated. Comparative tests on the susceptibility of other pathogenic rickettsiae were not performed.

MATERIALS AND METHODS

(1) *Seed suspensions*

The Henzerling strain of *R. burneti* was used and seed was made by emulsifying heavily infected yolk sacs to a 20 % w/v suspension in 10 % horse serum broth (used as diluent throughout the experiments). After light centrifugation the middle layer was distributed in 0·5 ml. quantities and sealed in glass ampoules. These were stored at − 70° C. and there was no evidence of loss of infectivity for the duration of the experiments.

(2) *Chemical agents*

Lysol B.P., commercial formalin (40% formaldehyde), phenol and anaesthetic ether, were obtained from ordinary laboratory sources. 'Dettol' is made by Reckitt and Colman Ltd., and is a solution of para-chlor-meta-xylenol. 'Cetavlon' is a quaternary ammonium compound (cetyltrimethyl ammonium bromide) with a detergent, manufactured by Imperial Chemical Industries (Pharmaceuticals) Ltd.

(3) *Fertile eggs*

Fertile White Leghorn hen's eggs were incubated at 38° C. for 6 days before inoculation of 0·25 ml. quantities into the yolk sacs. Subsequent incubation was at 36° C. with daily candling. From the 5th to the 14th day inclusive after inoculation, yolk sacs of all dead embryos were examined for rickettsiae using Giemsa stained impression smears. On the 14th day, the remaining eggs were also opened and examined. All eggs which showed indubitable rickettsiae on the 14th day or at earlier death, were counted as positive. Eggs surviving to the 14th day without clearly identified rickettsiae were called negative. When all the eggs in a group were negative, yolk sacs from the first six eggs were pooled in a 10 % suspension and passaged to a fresh batch of 6-day old eggs. If these were also negative, a further sub-passage was carried out in the same way.

All eggs in which embryos died in the first 4 days, and the few in which embryos died subsequently without detectable rickettsiae, were assumed to have succumbed to other causes and they were not counted in the experiment.

(4) *Experimental animals*

Laboratory bred guinea-pigs weighing 400–600 g. were inoculated intraperitoneally with 2 ml. quantities. Laboratory bred adult mice of the Glaxo *FF* strain received 0·25 ml. of inoculum intraperitoneally. Neither mice nor guinea-pigs were tested for Q fever antibody before inoculation, but sera from a considerable number of stock mice and guinea-pigs had been tested in previous experiments and none of them had demonstrable complement-fixing antibody at dilutions of 1:5.

The animals were killed 21 or 35 days after inoculation and specimens of serum from each were examined by the complement-fixation test for Q fever antibody. In preliminary experiments the final antibody titres were determined for each specimen of serum. A titre of 1:10 was taken to indicate infection in guinea-pigs and, as mouse sera were sometimes slightly anticomplementary at 1:5, a titre of 1:20 was taken as evidence of infection in the mice. In later experiments, sera were only tested at these critical dilutions. Specificity was checked by testing each serum with a normal egg antigen as well as the *R. burneti* antigen.

RESULTS

(1) *Infectivity titrations*

In attempts to find out the most sensitive technique for detecting small numbers of rickettsiae, seed suspensions were titrated for infectivity in guinea-pigs, mice and fertile eggs. Serial ten-fold dilutions of two seed suspensions (A and B) were made in 10 % horse serum broth, and batches of guinea-pigs, mice and fertile eggs were inoculated with each dilution.

The methods already described were used to test for infection of these animals and eggs. In Figs. 1 and 2 the results of several experiments are combined to show proportions of infected animals or eggs; the total numbers involved are given for each group. The infectivity titres are expressed as the 50% infectivity dose by the method of Reed & Muench (1938).

In the titration of seed A (Fig. 1) the animals were killed at 21 or 35 days after inoculation in order to find out if there was any delay in antibody formation when small infective doses were given. From this it appears that mice are more sensitive than guinea-pigs to infection with small numbers of rickettsiae especially if the mice are not examined for antibody until 35 days after inoculation. The mouse titrations also had much sharper end-points than those in guinea-pigs, and in fact there was considerable variation in the susceptibility of the guinea-pigs, some of which remained uninfected with seed diluted 10^{-5} and so were apparently insusceptible to at least 1000 mouse infective units. Titration in fertile eggs gave an I.D. 50 of $10^{-7.5}$ which showed that they were intermediate in susceptibility, but when the yolk sacs from the apparently uninfected eggs which had been inoculated with higher dilutions of seed were passaged to further fertile eggs, it was found that the sub-passage eggs all yielded rickettsiae, thus showing that a few organisms at least must have been present in the original eggs even when inoculated with a 10^{-11} dilution of seed.

For titrations of seed B the guinea-pigs and mice were all bled and tested at 35 days only (Fig. 2). In this experiment the mice did not appear to be more sensitive than guinea-pigs or first passage eggs. Again, however, passage of the eggs

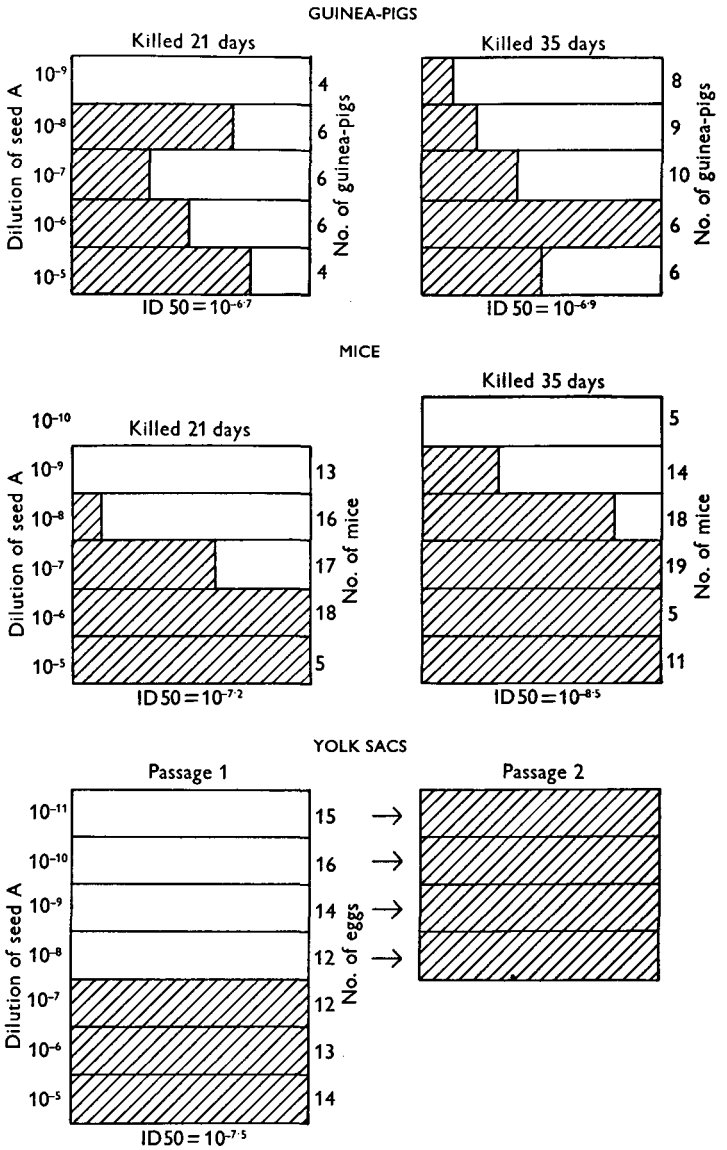

Fig. 1. Infectivity titrations of Henzerling seed A in guinea-pigs, mice and fertile hens' eggs. The shaded portions of the figure show the proportions of animals or eggs infected after inoculation with increasing dilutions of seed. The number opposite to each block gives the actual number of animals or eggs used.

inoculated with high dilutions of seed B showed rickettsiae to be present in dilutions even as high as 10^{-11}. Unfortunately, higher dilutions of seeds A and B were not tested.

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In order to check the possibility of antibodies developing in the inoculated guinea-pigs and mice against normal egg antigens, normal yolk-sac suspension was also titrated from 10^{-2} to 10^{-8} by inoculation into groups of these animals in a similar manner. When the guinea-pigs and mice were killed at 21 or 35 days no antibody could be detected against *R. burneti* antigen or normal egg antigens, even in the group which had received normal yolk sac diluted 10^{-2}.

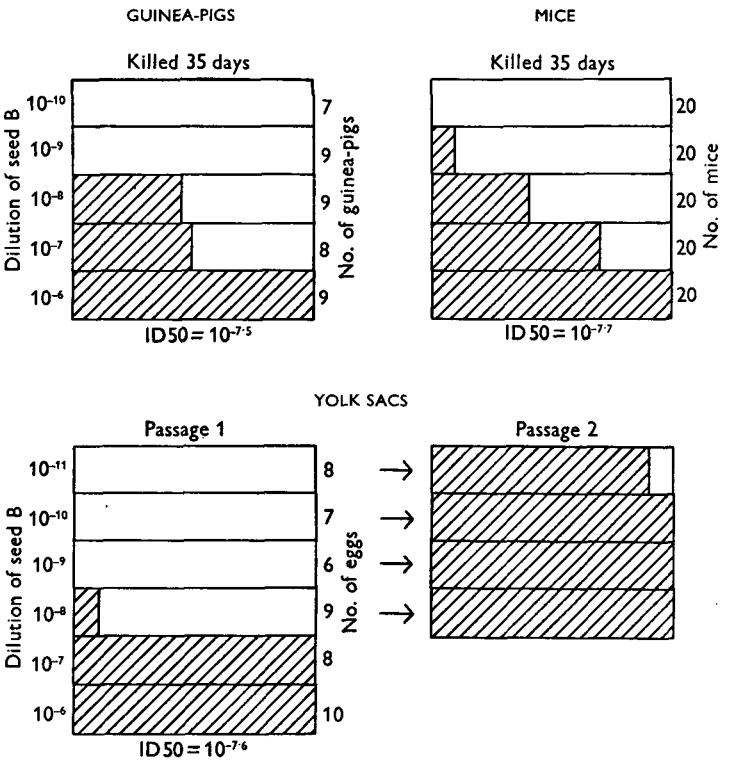

Fig. 2. Infectivity titrations of Henzerling seed B in guinea-pigs, mice and fertile hens' eggs. The shaded portions of the figure show the proportions of animals or eggs infected after inoculation with increasing dilutions of seed. The number opposite to each block gives the actual number of animals or eggs used.

It was concluded that inoculation of fertile eggs was the most suitable method for detecting small numbers of rickettsiae, provided that the apparently uninfected eggs were checked by sub-passage. This technique, with two sub-passages, was used for detecting viable rickettsiae after treatment with chemical agents.

The use of fertile eggs has the additional advantage that when inoculated with larger numbers of organisms, the length of survival of the embryo is related to the number of infective units. This is demonstrated in Table 1 and Fig. 3 which show the mean survival of embryos after inoculation of seed B diluted 10^{-3}, 10^{-4}, or 10^{-5}. This seed was used for all the tests with chemical agents so these figures are used as controls to give some indication of the quantitative effect of the agents on infectivity.

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Table 1. *Titration of seed suspension of* R. burneti *in fertile hens' eggs, showing day of death of embryos inoculated with dilutions of* 10^{-3}, 10^{-4} *and* 10^{-5}

Dilution of seed suspension	Exp. no.	No. of embryos dying on specified days after inoculation 7	8	9	10	11	12	13	14	15*	Mean day of death	Mean day of death for all exp.	Standard deviation of mean	Standard error of mean
10^{-3}	1	2	1	3	3	1	—	—	—	—	9·0			
	2	—	4	1	2	—	—	—	—	—	8·7	8·9	±1·04	0·21
	3	—	3	3	3	—	—	—	—	—	9·0			
10^{-4}	2	—	—	—	1	2	—	—	—	—	10·6	11·2	±1·02	0·28
	3	—	—	—	2	4	2	2	—	—	11·4			
10^{-5}	2	—	—	—	—	—	1	1	3	—	13·4	13·8	±0·76	0·19
	3	—	—	—	—	—	—	3	7	2	13·9			

* Two out of twelve embryos inoculated with 10^{-5} (exp. 3) were alive on the 14th day, when the experiment was erminated to avoid hatching. For the purposes of calculation these are counted as dying on the 15th day.

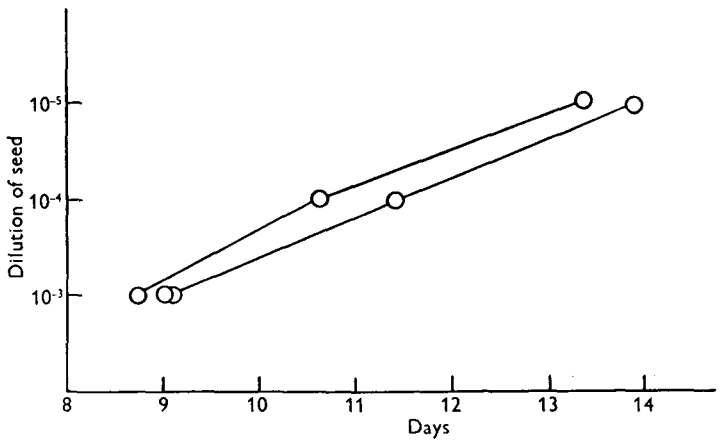

Fig. 3. Mean day of death of groups of fertile hens' eggs inoculated with increasing dilutions of Henzerling strain of *R. burneti* (seed B).

Fertile eggs are not suitable for primary isolation of new strains of *R. burneti*, because they are too susceptible to bacterial contaminants. Since mice appeared to be as sensitive to *R. burneti* as guinea-pigs in the titrations of an egg adapted strain, we also compared the susceptibility of these animals to strains of *R. burneti* from cows. Two naturally infected milk pools, collected at dairies, were therefore titrated by making serial ten-fold dilutions in sterile skimmed milk and then inoculating groups of guinea-pigs and mice as before. The results given in Fig. 4 show that, compared to guinea-pigs, mice are relatively less susceptible to the milk strains, but this might be remedied in practice by using larger numbers of mice, which are generally easier to obtain than guinea-pigs.

(2) Rickettsia burneti *in vaccine lymph*

Because the matter was urgent, the first chemical to be investigated was phenol. The problem was to determine whether *R. burneti* contaminating crude vaccine pulp harvested from sheep, would survive the routine treatment with 1·0 % phenol and with glycerol, as used in the preparation of vaccine lymph in Great Britain

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(McClean, 1949). It seemed advisable to test this by mixing live *R. burneti* with crude vaccine pulp and then subjecting the mixture to the methods of routine purification which are used in the Lister Institute.

A suspension of *R. burneti* in the form of a 1:1000 final dilution of seed B was ground thoroughly with crude vaccine pulp harvested from sheep* together with 2 ml. of 1 % phenol per gram of pulp. The ground suspension was allowed to stand at 22° C. for 48 hr. and then diluted with 2 ml. of glycerol per gram. This reduced the concentration of phenol to about 0·4 %. After sieving through a wire mesh the final preparation was stored at −20° C. until tested. Control suspensions of *R. burneti*, *R. burneti* and phenol, *R. burneti* and vaccine pulp, and phenol with vaccine pulp, were run in parallel under identical conditions.

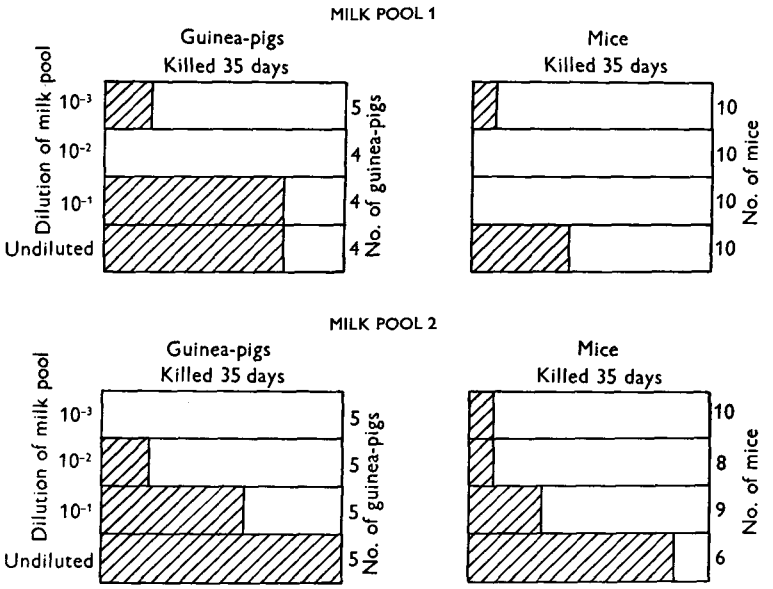

Fig. 4. Infectivity titrations of two naturally infected milk pools in guinea-pigs and mice. The shaded portions of the figure show the proportions of animals infected after inoculation with increasing dilutions of milk. The number opposite to each block gives the actual number of animals used.

Fertile eggs could not be used to test for living *R. burneti* in this experiment because the embryos would have died quickly of generalized vaccinia. The preparations were therefore inoculated into groups of two or three guinea-pigs because at that time the susceptibility of mice was not fully realized. The normal criterion for infection in the guinea-pig is a rise in specific antibody titre, but rickettsiae killed by phenol might produce such a response. All the guinea-pigs were therefore killed 18 days after inoculation and 10 % suspensions of liver, spleen and kidney from each animal were passed separately to further guinea-pigs. If a rise in Q fever antibody titre was detected in the animals of the second passage it was assumed that living rickettsiae had been present in the animals of the first passage.

* The crude vaccine pulp was kindly supplied by Dr Douglas McClean, of the Lister Institute.

The results which are shown in Table 2 indicate that in the presence of 1 % phenol, glycerol and vaccine lymph the infectivity of *R. burneti* is not destroyed. After treatment with 1 % phenol and glycerol without vaccine lymph no living organisms were isolated. It is possible that the presence of the crude vaccine pulp diminishes the actions of these agents.

It therefore appears that *R. burneti* contaminating crude vaccine pulp in moderate doses would survive in the final purified vaccine lymph. The relative insusceptibility of the organism to phenol is confirmed in the experiments described in the next section.

Table 2. *Effect of adding* R. burneti *to crude vaccine pulp from sheep. The vaccine pulp was then subjected to the standard purification procedure with phenol and glycerol. The final suspension was tested for living* R. burneti *by two passages in guinea-pigs*

	First passage		Second passage	
Inoculum	Guinea-pig no.	C.F.T. 18th day (killed for passage)	Guinea-pig no.	C.F.T. 35th day
R. burneti	1	+	1A	−
Vaccine pulp			B	+
Phenol	2	−	2A	−
			B	−
	3	+	3A	+
			B	−
R. burneti	4	+	4A	+
Vaccine pulp			B	+
	5	+	5A	+
			B	−
R. burneti	6	+	6A	−
			B	−
	7	+	7A	+
			B	+
	8	+	8A	+
			B	−
R. burneti	9	−	9A	−
Phenol			B	−
	10	−	10A	−
			B	−
	11	−	11A	−
			B	−
	12	−	12A	−
			B	−
	13	−	13A	−
			B	−
	14	−	14A	−
			B	−
Vaccine pulp	15	−	15A	−
Phenol			B	−
	16	−	16A	−
			B	−

+ = Complement fixing antibodies to *R. burneti* present at 1:10 or higher.
− = No complement fixing antibodies to *R. burneti* present at 1:10.

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(3) *Susceptibility to chemical disinfectants*

The effect of the various chemical agents was determined on Henzerling strain seed B. The final thousandfold dilution which was used represented about $10^{4.5}$ I.D. 50 for eggs on the basis of one passage, but a much higher infective dose if blind sub-passages were made. (See Results, §1, p. 504.)

When chemicals were used at concentrations of less than 0·5 % they were added so as to give the desired concentration in the standard 1:1000 dilution of seed suspension. After action for the necessary time and temperature, this mixture was then inoculated directly into eggs. As concentrations of the chemicals of 0·5 % or over caused toxic deaths of the embryos some dilution was necessary before introduction into the eggs. When the chemicals were used at 0·5 %, therefore, they were first mixed with twice the standard concentration of seed (i.e. 1:500). After the time allowed for action the mixtures were then diluted so as to give the standard 1:1000 concentration of original seed with the agent at a non-toxic strength of 0·25 %. Chemicals used at 1 % were mixed with a 1:250 dilution of seed suspension and finally diluted four-fold before introduction into the eggs. This meant that various concentrations of chemicals were acting on different strengths of seed suspension but the effects were made comparable by restandardizing in terms of original seed before finally inoculating into eggs. Once in the yolk sac it is unlikely that the chemicals continued to act, because of their further dilution.

In most experiments the chemicals were allowed to act for 3 hr. at 22° C. This rather short period of action was tested because chemicals used for disinfection of laboratory glassware, benches, etc., are rarely allowed to act for long rigidly prescribed times and it was thought better to test a minimum rather than a maximum period. It was also for purely practical reasons that most tests were done at 22° C. which is near the room temperature at which these agents would usually be acting. Nevertheless, the effects of higher and lower temperatures were also investigated.

Table 3 shows the results of tests with phenol, formalin, 'Cetavlon', 'Dettol' and 'Lysol', acting in various concentrations for 3 hr. at 22° C. It will be observed that eggs inoculated with the control, untreated, suspension had a mean survival of 8·9 days (see also Table 1). The increase in survival time of eggs inoculated with treated suspension is an indication of the influence of the chemical used. From the titration of this seed (Table 1) it will be observed that an increase in survival of about 2·5 days indicates a ten-fold fall in infectivity.

On this basis phenol had little effect if used in a concentration of 0·5 % or less. With 1 % phenol there was approximately a thousand-fold reduction in infectivity, but every egg was still infected.

The quaternary ammonium compound 'Cetavlon' at 0·5 % had a slightly greater effect than phenol but the action was not much increased at the higher concentration of 1 %. However, it will be seen from the standard error that there was a considerable spread in survival times of the 1 % 'Cetavlon' group so that the mean figure of 12·0 does not carry a high significance.

Formalin was more effective because a concentration of 0·5 % reduced infectivity considerably, while suspensions treated with 1 % formalin caused infection in only two of thirteen eggs (in one passage).

The effect of 'Dettol' was much the same as that of formalin. 'Lysol' however, was much more potent. In one egg passage no organisms were detected after treatment of the seed suspension with 0·5 % or 1 % 'Lysol', but the apparently uninfected eggs of the first passage contained viable rickettsiae, shown by sub-passage to further eggs. Nevertheless, the numbers of living organisms must have been very small indeed, especially after treatment with 1 % 'Lysol', because two blind sub-passages were needed to show their presence.

Table 3. *Effect of five chemical agents acting in various concentrations for* 3 *hr. at* 22° *C. upon a suspension of* R. burneti. *The treated suspension was tested for viable organisms by inoculation of fertile hens' eggs*

Agent	Conc. %	No. of eggs inoculated	No. infected	Mean day of death, post-inoculation (with standard error), or result of sub-passage	Increase in mean day of death over controls
Phenol	0·1	25*	25	9·6 (± 0·22)	0·7
	0·25	16	16	8·7 (± 0·28)	Nil
	0·3	34*	34	10·3 (± 0·15)	1·4
	0·5	8	8	8·8 (± 0·25)	Nil
	1·0	8	8	Survived	
'Cetavlon'	0·5	14	14	11·2 (± 0·49)	2·3
	1·0	16	16	12·0 (± 0·58)	3·1
Formalin	0·25	15	15	11·5 (± 0·36)	2·6
	0·5	11	11	13·5 (± 0·33)	4·6
	1·0	13	2	Survived	
'Dettol'	0·5	8	8	Survived	
	1·0	17	7	Survived	
'Lysol'	0·5	12	0	Pass. 2 positive	
	1·0	15	0	Pass. 2 negative Pass. 3 positive	
Control (no agent)	—	26	26	8·9 (± 0·2)	

* Pooled results of two experiments.

It was disturbing to find that 0·5 % formalin was only partially effective because it is routinely used to destroy infectivity of suspensions before antigen and vaccine preparation. However, the usual procedure is to leave the crude suspension of yolk sacs in the presence of 0·5 % formalin for 48 hr. at + 4° C. We therefore tested such a suspension which was awaiting processing to antigen in our laboratory. Living rickettsiae were isolated without difficulty in one egg passage. Most techniques of antigen and vaccine production subsequently involve treatment with ether. With *R. burneti* the purpose of this is simply to effect a separation of rickettsial particles from tissue debris. Since formalin was not effective it was important to find out if ether destroyed the infectivity of the organisms. Accordingly an equal volume of ethyl ether was shaken by hand for 1 min. with 1:1000 seed B, and the mixture was then allowed to separate by standing overnight at 4° C. Next morning the aqueous layer containing the rickettsiae was withdrawn and inoculated into eggs. No organisms were found in these or on eggs of the first

sub-passage. Eggs of the second sub-passage, however, were all infected. Thus treatment with ether was not completely effective, but it left very few viable organisms.

Further experiments were carried out to determine the effect of temperature upon the action of some of these chemical agents on *R. burneti*. It was first necessary to ensure that untreated control suspensions suffered no deterioration of infectivity under the conditions of the test. From the data in Table 4 it will be seen that there is in fact no serious loss after seed is left 3 hr. at 4, 22 or 37° C. or even for 48 hr. at 4 or 22° C.

Table 4. *Effect of holding seed suspension of* R. burneti, *at* 4, 22 *and* 37° *C., before inoculation of fertile eggs*

Temperature ° C.	Time exposed in hours	No. of eggs inoculated	No. of eggs infected	Mean day of death post-inoculation (with standard error)
4	3	5	5	10·2(±0·36)
	48	7	7	9·0(±0·31)
22	3	8	8	10·0(±0·36)
	48	6	6	9·5(±0·32)
37	3	4	4	9·25(±0·6)
Control inoculated immediately		10	10	9·2(±0·21)

Table 5. *Effect of temperature on the action of formalin, 'Dettol' and 'Lysol' acting for* 3 *hr. upon a suspension of* R. burneti. *The treated suspension was tested for viable organisms by inoculation of fertile hens' eggs*

Agent	Conc. %	Temperature of action ° C.	No. of eggs inoculated	No. of eggs infected	Mean day of death post-inoculation (with standard error), or results of sub-passage
Formalin	0·25	22	15	15	11·5(±0·36)
		37	9	0	Pass. 2 positive
	0·5	4	17	17	11·8(±0·46)
		22	11	11	13·5(±0·33)
		37	8	0	Pass. 2 negative Pass. 3 positive
'Dettol'	1·0	4	7	0	Pass. 2 positive
		22	8	0	Pass. 2 positive
		37	6	0	Pass. 2 positive
'Lysol'	1·0	22	15	0	Pass. 2 negative Pass. 3 positive
		37	4	0	Pass. 2 and 3 negative
Control	—	—	26	26	8·9(±0·2)

The effect of formalin, 'Dettol' and 'Lysol' allowed to act for 3 hr. at 4, 22 and 37° C. is seen in Table 5. With formalin the rise in temperature enhances the action markedly so that 0·5 % formalin allowed to act at 37° C. almost completely removes infectivity, detectable only after two sub-passages. However, with

'Dettol' used at higher concentration the effect was less obvious. As we have seen, 1 % 'Lysol' is very effective even at 22° C. After action at 37° C. no living rickettsiae could be isolated at all, even after two sub-passages. This was the only completely successful disinfection which we accomplished.

DISCUSSION

The infectivity titrations show that with one passage fertile eggs are about as susceptible as mice or guinea-pigs to small numbers of viable *R. burneti*. Nevertheless, it is possible to increase the sensitivity of the egg technique enormously by carrying out blind sub-passages from apparently uninfected eggs. This may simply be due to the increased time available for growth which can thus continue up to 42 days in the absence of antibody. In any case, the high susceptibility of eggs to an egg-adapted strain is not surprising.

The guinea-pigs used in our experiments showed considerable variation in susceptibility to this strain and they were therefore less satisfactory than mice for infectivity titrations. This may be due to the fact that the guinea-pigs used were a mixed breed, whereas the mice were from a pure line. However, in titration of naturally occurring strains from milk the mice were no more susceptible than the guinea-pigs.

R. burneti showed considerable resistance to the common laboratory disinfectants which were tested. In strengths up to 1 % neither phenol, formalin, 'Cetavlon', 'Dettol' nor 'Lysol' destroyed infectivity completely, after 3 hr. at 22° C. By determining the prolongation of survival of embryos inoculated with treated rickettsiae it was possible to make some comparative assessment of the disinfecting action of these chemicals. Under the conditions of testing, phenol was the least active because a 0·5 % concentration had no measurable effect on *R. burneti*. 'Cetavlon', formalin and 'Dettol', were increasingly effective in that order, but 'Lysol' was easily the most active disinfectant. Few viable organisms remained after treatment with 1 % 'Lysol' for 3 hr. at 22° C. Increased temperature enhanced the action of 'Lysol' and in fact the only complete disinfection was by 1 % 'Lysol' acting at 37° C. This suggests that warm 'Lysol' in concentrations of 1 % and higher is a satisfactory general disinfectant for laboratories in which work with *R. burneti* is being done.

After misplaced reliance on formalin for the destruction of infectivity in Q fever vaccines and antigens, it is comforting to know that ether is highly effective, but it is apparent that a few viable organisms can survive even this treatment. It is thus possible that a few living rickettsiae may be present in some Q fever vaccines, and escape detection at the final infectivity check, unless several blind egg passages are carried out. However, it is doubtful if this faint possibility constitutes any danger to vaccinated individuals. Immunologically, it might even be an advantage.

The failure of 1 % phenol to kill *R. burneti* means that viable rickettsiae harvested with crude vaccine pulp from sheep, could survive in the final purified vaccine lymph. Sheep are the principal reservoirs of Q fever in many parts of the world, and it is thought that *R. burneti* can persist for long periods in the wool when it has been contaminated with infected placental fluid or faeces.

It should be noted that the same considerations also apply to calves which are used for vaccine lymph production in many countries. Preliminary tests for Q fever antibody are now being performed on sheep used at the Lister Institute and might well be carried out on all animals to be used for vaccine production in countries where Q fever is endemic, but adequate shaving and cleaning of the skin should reduce possible contamination of vaccine lymph very greatly.

The chemical agents investigated by Ransom & Huebner (1951) were allowed to act on *R. burneti* at 4° C. for longer periods than those used by ourselves, but the results described in this paper generally agree with theirs. Ransom & Huebner did not isolate viable rickettsiae after treatment with ether but only one egg passage was used.

SUMMARY

1. The susceptibility of the Henzerling strain of *R. burneti* to phenol, formalin, 'Lysol', 'Dettol', 'Cetavlon', and ether was investigated.

2. Preliminary investigation of techniques showed that, with one passage, guinea-pigs, mice and fertile hens' eggs were of similar susceptibility to small numbers of viable organisms, but the greater feasibility of sub-passage from the eggs increased the sensitivity and made this the technique of choice.

3. Under the condition of testing, 'Lysol' was the most active disinfectant. Treatment of the rickettsial seed with 1 % 'Lysol' at 37° C. was in fact the only effective method for destroying completely the infectivity in 3 hr.

4. Some living organisms survived contact for 3 hr. with each of the other chemicals in concentrations up to 1 %. A rough quantitative comparison of these agents was made.

5. The failure of 1 % phenol to destroy *R. burneti* raised the possibility of this organism surviving as a contaminant in vaccine lymph. This problem was investigated and is discussed.

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