BACTERIAL SURVIVAL IN SYSTEMS OF LOW MOISTURE CONTENT

PART II.—THE BACTERICIDAL EFFECTS OF CERTAIN SUBSTANCES DURING THE SPRAY DRYING PROCESS

BY KENNETH BULLOCK AND E. A. RAWLINS

From the Department of Pharmacy, University of Manchester

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INTRODUCTION

IN previous communications from this Department[1,2] the production of spray-dried bacterial cultures has been described, and some of the effects of heat and of storage on these cultures have been examined. It has been shown that *Bacterium lactis aerogenes*, a non-sporing organism, suffers a high mortality on spray-drying, and that the surviving organisms rapidly lose their viability. On the other hand, the spores of *Bacillus subtilis* were not killed during the drying process and maintained their viability on storage. It was thought that the addition of a bactericide to the bacterial suspension before it was dried might result in the production of a sterile powder and, as the concentration of bactericide would rapidly be raised during the drying process, it appeared that sterility of the powder might be obtained by the use of very small initial concentrations of bactericide. Moreover, if a volatile bactericide were used, much or all of it might be volatilised during drying so that the resultant powders would contain traces at most. Such a process should be suitable for the production of injections and food products, and the process would be very useful for the sterilisation of thermolabile substances, as these have been shown to undergo no decomposition when spray-dried[3]. The experiments described in this paper show, however, that very few substances are capable of producing sterility when used for the above purpose, but the diversity of the results obtained prompts further inquiry. A critical examination has therefore been made of the factors involved when micro-organisms are spray-dried in the presence of potentially toxic substances and the significance of the observed results has been discussed.

EXPERIMENTAL TECHNIQUE

The Test Organism. When testing a process to ascertain if it can bring about sterility it is necessary to impose upon it the severest possible conditions. The experiments described in the present communication have therefore been performed using an organism known to exhibit a high resistance to disinfectant processes. For this purpose a suspension of spores of *B. subtilis* (Marburg) was prepared as previously described[2].

Preparation of Spray-dried Powders. The technique of preparing spray-dried powders containing micro-organisms has already been described[1,2]. In the earlier experiments presented in the present paper peptone-water was used as the substrate on which to dry the bacteria.

This was prepared as before[2] and inoculated with sufficient of the suspension of the test organism to give an expected count of 2×10^5 organisms per ml. The suspension was fed to the dryer and the resultant powder collected. It is known, however, that protein matter reduces the disinfectant action of some phenolic compounds[4] and of some other substances. Moreover, during the later stages of the drying process it might be expected that the organisms would become coated with a continuous film of the colloidal material which would reduce the effectiveness of gaseous bactericides. Some of the experiments were therefore repeated using lactose solution as the substrate in which to suspend the bacteria. The utility of lactose, however, is limited by the fact that at 130°C. it loses water of crystallisation and becomes hygroscopic, while at higher temperatures it melts. It is difficult, therefore, to prepare a spray-dried lactose powder which is free from stickiness and which can be sampled satisfactorily. In later experiments it was found that the spores could be dried using a solution of sodium acid phosphate as substrate, without suffering any significant mortality. The resultant powder was mobile and rather dense, making it easy to handle, the concentration of its solutions can be estimated chemically and it undergoes complete dehydration in the dryer. Several experiments were therefore carried out using sodium acid phosphate as substrate. Prior sterilisation of the various substrates was effected by autoclaving or, where this was inapplicable, by filtration. Non-volatile solid bactericides were added to the substrate before sterilising it, while volatile and liquid substances were added after sterilisation and immediately before drying, as also was the suspension of spores.

Reconstitution of the Bacterial Suspension and Estimation of Mortality During Drying

In order to estimate the mortality occurring during drying, portions of the spray-dried powder were dissolved in sterile water and viable counts were performed on the original and reconstituted suspensions. The relative strengths of the two suspensions were then compared by estimating their content of one non-volatile component. The following methods of estimation were used:—

(a) Peptone was estimated as before[2].

(b) In the case of lactose 0·001 per cent. of ferrous sulphate was incorporated in the original suspension and the iron was estimated colorimetrically by means of ammonium thioglycollate.

Suspensions containing sodium sulphate were similarly estimated.

(c) The B.P. assay processes were used for estimating sodium chloride, sodium phosphate, sodium acid phosphate, sodium nitrite, sodium carbonate, potassium chloride, and potassium chlorate, and the B.P.C. method for estimating calcium formate.

Potassium bromide was estimated by the method used for sodium chloride.

(d) In the case of the solution containing lactic acid and sodium acid phosphate the latter was converted to the magnesium ammonium salt which was filtered off and ignited and the pyrophosphate weighed.

Method of Performing Viable Counts. Roll-tube counts were performed using graduated pipettes for mixing and measuring the bacterial suspensions. The technique employed was that previously described and tested[2]. In most cases viable counts were performed on 10^{-3} dilutions of the original and reconstituted suspensions, though in some of the earlier experiments lower dilutions were used. In all cases, however, the bactericides present were diluted sufficiently to ensure that they would have no inhibitory effect on the growth of the organisms in the culture medium.

THE SPRAY-DRYING OF ORGANISMS WITH BACTERICIDES

Terminology. While the presence of viable organisms in a liquid or powder cannot be disproved without examining the whole of the material, the evidence provided by viable counts performed on small samples is sufficient to establish that the degree of contamination is low enough to be regarded as insignificant. The term " sterility " is, therefore, used in this paper to indicate that no viable organisms have been demonstrated by means of roll-tube counts.

The term " mortality " is used to represent the fall in viable count during spray-drying. It is estimated as a percentage of the number of organisms present in the original suspension.

The " inlet temperature " is the temperature of the air entering the main chamber of the drier.

The " substrate " is the substance, or mixture of substances, which, occurring in high concentration in the original suspension fed to the drier, forms the main bulk of the spray-dried powder.

The Effect of Drying on Salt Substrates. In our earlier experiments[2] the organisms were dried suspended in plain peptone water containing no added sodium chloride, as it was thought that this might itself exert a lethal effect on the organisms in the later stages of the drying process, since the concentration of the salt would then be high. An examination of the effect of drying on a substrate of sodium chloride alone was therefore made. Using a 5 per cent. solution of sodium chloride as substrate and inlet temperatures of 190°C. a mortality of about 50 per cent. was obtained. A 2 per cent. solution gave a slightly lower mortality. The 5 per cent. solution was also dried using a lower inlet temperature in order to increase, if possible, the period of contact of the spores with the concentrated solution of the salt: this resulted in a slightly higher mortality of 60·5 per cent. Raising the inlet temperature to 301°C. resulted in a 99·1 per cent. mortality. Since it is known[5] that salts vary in their bactericidal effect, both anions and cations showing specific activity, further dryings were carried out using a number of different salts as substrates. The results of these experiments are given in Table I.

Three different halides were tested, sodium chloride, potassium chloride and potassium bromide, and there appears to be no significant difference between the mortalities they produced. Sodium sulphate produced a much lower mortality than the halides, possibly owing to the

TABLE I

Substrate	Inlet temperature	Mortality per cent.
Sodium chloride, 2 per cent.	198°C.	41·5
,, ,, 5 ,, ...	191°C.	54·8
,, ,, ,, ,, ...	190°C.	45·2
,, ,, ,, ,, ...	85°C.	60·5
,, ,, ,, ,, ...	301°C.	99·1
Potassium chloride, 2·56 per cent.	195°C.	53·3
Potassium bromide, 4·0 per cent.	185°C.	48·5
Sodium sulphate, 4·80 per cent....	191°C.	20·5
Sodium dihydrogen phosphate, 5·34 per cent....	188°C.	3·8
Disodium hydrogen ,, 12·2 per cent....	185°C.	6·6
Sodium carbonate, 2·42 per cent.	200°C.	68·4
Sodium nitrite, 2·36 per cent.	187°C.	98·3
Potassium chlorate, 4·19 per cent.	200°C.	77·8
Boric acid, 3·00 per cent....	200°C.	60·2
Calcium formate, 4·45 per cent....	200°C.	73·6
Sodium chloride, 5 per cent., and polyoxyethylene stearate, 0·1 per cent.	202°C.	16·3
Sodium dihydrogen phosphate, 2 per cent., and sodium chloride, 2 per cent.	180°C.	27·4
Sodium thiosulphate, 5 per cent....	192°C.	38·1

formation of the anhydrous salt, which has a limited solubility at temperatures above 33°C. Oxidising and reducing agents are known to be toxic to bacteria, and potassium chlorate, sodium nitrite and calcium formate all produced fairly high mortalities. Boric acid and sodium carbonate produced mortalities of the same order, while sodium thiosulphate proved to be less toxic. It is interesting to note that when the spores were dried with sodium chloride as substrate in the presence of a polyoxyethylene stearate the spores were protected against the lethal action of the halide. This may be because the polyoxyethylene stearate is a waxy solid and on drying it forms a protective coating over the organisms. The most interesting result, however, is that of drying the spores on a phosphate substrate. Both sodium acid phosphate and sodium phosphate, alone among the salts tested, produced no mortality. Further work is being undertaken to elucidate the significance of this.

Phenolic Compounds. The mortalities produced by spray-drying the spores with various phenolic substances are recorded in Table II. In the work previously published, mortalities have been demonstrated varying between −15 per cent. and +12 per cent. when spray-drying *B. subtilis* spores in peptone water in the absence of antiseptics. The number of observations was too small to provide an accurate index of variation, but it would appear unwise to consider any mortality of less than 15 per cent. to be significant. It is evident, therefore, that both phenol and chlorocresol are ineffective in the presence of peptone and also when dried with lactose.

Resorcinol gives the highest mortality exhibited by the pure phenols, a finding in line with that of Twort and Baker[6], who found resorcinol to be the most effective phenol of those they tested for activity in bactericidal mists. Phenylmercuric nitrate 0·002 per cent. shows about the same mortality as resorcinol 0·5 per cent.

TABLE II

Substrate	Bactericide	Inlet temperature	Mortality per cent.
Peptone, 4 per cent.	Phenol, 1 per cent.	165°C.	0·0
Peptone, 4 „	Phenol, 0·5 per cent.	178°C.	11·1
Peptone, 4 „	Phenol, 0·5 per cent.	178°C.	12·5
Peptone, 4 „	Chlorocresol, 0·2 per cent. ...	178°C.	6·9
Peptone, 4 „	Phenylmercuric nitrate, 0·002 per cent.	175°C.	32·0
Peptone, 4 „	Resorcinol, 0·5 per cent. ...	199°C.	29·6
Lactose, 4 „	Phenol, 0·5 per cent.	81°C.	16·2
Lactose, 4 „	Chlorocresol, 0·2 per cent. ...	148°C.	2·7
Lactose, 4 „	Phenylmercuric nitrate, 0·002 per cent.	155°C.	37·0
Sodium chloride, 2 per cent. ...	Phenol, 0·5 per cent.	200°C.	61·6
Sodium dihydrogen phosphate, 5 per cent.	Phenol, 0·5 „	185°C.	67·2

Surface-Active Substances. Surface-active agents may be classified as anionic, cationic, or non-ionic compounds. Studies of the bactericidal activity of the anionic and cationic compounds have been made by several workers[7,8,9], and some of the cationic compounds have been found very effective against Gram-positive organisms. Dunn[10], however, showed that their activity was greatly reduced by serum, and Baker, Harrison and Miller[7] found it to be influenced by *p*H. In Table III the results of spray-drying *B. subtilis* spores in the presence of surface active agents of each type are presented.

TABLE III

Substrate	Surface-active agent	Inlet temperature	Mortality per cent.
Peptone, 4 per cent.	Sodium lauryl sulphate, 0·5 per cent.	185°C.	4·6
Peptone, 4 „	CTAB,* 0·1 per cent.	191°C.	27·9
Lactose, 4 „	„ „	149°C.	32·1
Sodium dihydrogen phosphate, 5 per cent.	„ „	195°C.	87·9
Disodium hydrogen phosphate, 5 per cent.	„ „	194°C.	88·6
Lactose, 4 per cent.	Stergene, 0·5 per cent. ...	145°C.	6·6

* *Cetyltrimethylammonium bromide.*

Phenols Combined with Surface-Active Agents. Apart from their own germicidal activity, surface-active agents may increase the bactericidal effect of other substances by acting as solubilising or emulsifying agents and increasing the rate of penetration. Gershenfeld *et al.*[8] tested a number of detergents in conjunction with phenolic, mercurial and halogenated bactericides, and found that the effect of these was not increased. Valko and Dubois[9] showed that the bactericidal effect of cationic detergents could be nullified by anionic detergents, and Harper[11] has suggested that in order to obtain the maximum bactericidal effect an anionic detergent must always be used with an anionic bactericide and a cationic detergent with a cationic bactericide. Table IV shows the results obtained on spray-drying *B. subtilis* spores in the presence of phenolic disinfectants together with surface-active agents.

TABLE IV

Substrate	Surface-active agent	Bactericide	Inlet temperature	Mortality per cent.
Peptone, 4 per cent.	Sodium lauryl sulphate, 0·5 per cent. ...	Phenol, 0·5 per cent.	185°C.	26·1
,, ,,	,, ,,	Chlorocresol, 0·2 per cent....	180°C.	17·9
,, ,,	,, ,,	Phenylmercuric nitrate, 0·002 per cent. ...	180°C.	53·8
,, ,,	CTAB, 0·01 per cent.	Chlorocresol, 0·2 per cent....	188°C.	0·0
,, ,,	,, ,,	Phenol, 0·5 per cent.	191°C.	57·8
Sodium chloride, 2 per cent.... ...	Polyoxythylene stearate, 0·1 per cent.	,, ,, ...	200°C.	0·0

Glycols and Hydroxyacids. The use of glycols for the promotion of aerial disinfection has now become an established practice, chiefly owing to the work of Robertson and his associates[12,13]. Puck[14] showed that the activity of these compounds was due to condensation of vapour molecules on the bacteria-carrying particles, thus demonstrating the necessity for the complete vaporisation of glycols. These workers also showed that the maximum activity of the glycols was exerted in atmospheres the relative humidity of which lay between 45 and 70 per cent. De Ome[15] showed that triethylene glycol was 100 times more effective than propylene glycol against *S. pullorum*. Lidwell, Lovelock and Raymond[16] found lactic acid vapour to be an efficient aerial germicide, but with this also the bactericidal activity decreased rapidly in atmospheres having relative humidities below 50 per cent. Table V shows the results obtained when *B. subtilis* spores were spray-dried

TABLE V

Substrate	Bactericide	Inlet temperature	Mortality per cent.
Peptone, 4 per cent.	Ethylene glycol, 0·5 per cent.	185°C.	13·5
Sodium dihydrogen phosphate, 5 per cent.	Ethylene glycol, 1·0 per cent.	185°C.	29·0
,, ,, ...	Propylene glycol, 1·0 per cent.	200°C.	66·5
,, ,, ...	Triethylene glycol, 1·0 per cent.	192°C.	53·8
,, ,, ...	Polyethylene glycol, 1·0 per cent.	200°C.	39·2
,, ,, ...	Lactic acid, B.P., 1·0 per cent.	194°C.	88·7

in the presence of four members of the glycol series separately and also in the presence of lactic acid. The first drying was carried out using peptone as substrate, but thereafter it was found that sodium acid phosphate had no lethal effect on the spores and this was used for the remainder of the dryings in order to eliminate the protective action of the peptone colloids.

Lactic acid killed a high proportion of the spores and was superior in this respect to any of the glycols, but the maximum bactericidal activity of the latter was exerted by propylene glycol: triethylene glycol was less active, while the polyethylene glycol had less effect than triethylene glycol. Ethylene glycol was the least active of the series and showed a negligible activity in the presence of peptone.

Gaseous Antiseptics. Douglas, Hill and Smith[17] showed that sodium hypochlorite solution when sprayed into the air could, even in very high dilution, rapidly kill *Bact. coli,* and these findings were later confirmed by Masterman[18], who concluded that the activity was due to liberation of gaseous hypochlorous acid. Formaldehyde, too, has long been used for the disinfection of unoccupied rooms, and Salle and Korzenovsky[19] showed that it could be used for the sterilisation of materials contaminated with bacteria. These workers also found that ethylene oxide, which has been employed for the fumigation of clothes and seeds, shows low bactericidal activity. Table VI records the mortalities produced by varying concentrations of these substances when solutions of them were spray-dried with *B.* subtilis spores.

TABLE VI

Substrate	Bactericide	Inlet temperature	Mortality per cent.
Peptone, 4 per cent.	Chloroform, 0·5 per cent. ...	168°C.	16·4
,, ,, ...	Milton, 1·0 per cent.	190°C.	6·4
Sodium dihydrogen phosphate, 5 per cent.	,, 0·1 ,, ...	198°C.	100·0
Sodium dihydrogen phosphate, 5 per cent.	,, 0·01 ,, ...	200°C.	20·8
Disodium hydrogen phosphate, 5 per cent.	,, 0·1 ,, ...	196°C.	47·9
Peptone, 4 per cent.	Formalin, B.P., 2·5 per cent....	175°C.	100·0
,, ,, ...	Formalin, B.P., 2·0 per cent....	195°C.	100·0
,, ,, ...	Formalin, B.P., 1·0 per cent....	195°C.	100·0
,, ,, ...	Formalin, B.P., 0·75 per cent....	185°C.	97·0
,, ,, ...	Formalin, B.P., 0·5 per cent....	190°C.	74·3
,, ,, ...	Formalin, B.P., 0·25 per cent....	193°C.	48·5
,, ,, ...	Formalin, B.P., 0·05 per cent....	185°C.	3·0
,, ,, ...	Formalin, B.P., 0·005 per cent.	193°C.	0·0
Lactose, 4 per cent.	Formalin, B.P., 0·5 per cent....	159°C.	100·0
,, ,, ...	Formalin, B.P., 0·05 per cent....	155°C.	100·0
,, ,, ...	Formalin, B.P., 0·005 per cent.	155°C.	67·1
Sodium dihydrogen phosphate, 5 per cent.	Ethylene chlorhydrin, 0·5 per cent.	185°C.	14·4
,, ,, ...	Ethylene oxide, 1·0 per cent....	190°C.	58·8
Disodium hydrogen phosphate, 5 per cent.	,, ,, ...	190°C.	41·8

The sodium hypochlorite solution employed (Milton) contained 1 per cent. of sodium hypochlorite: a 0·1 per cent. dilution of this solution produced complete sterility when sodium acid phosphate was used as substrate, while with sodium phosphate it produced a mortality of 47·9 per cent. In the presence of peptone the hypochlorite was completely inactivated, even when using a 1 per cent. dilution. Formaldehyde was used in the form of Liquor Formaldehydi B.P. Using lactose as substrate a 0·05 per cent. dilution of Liquor Formaldehydi was sufficient to produce sterility, and even a 0·005 per cent. dilution produced a mortality of 67 per cent. Formaldehyde showed the greatest bactericidal activity of any of the substances tested. As might be expected, it was inactivated to a considerable extent by peptone, but in the presence of this a 1 per cent. dilution still produced a 100 per cent. mortality. Ethylene oxide killed 58·8 per cent. of the spores when they were dried on sodium acid phosphate. Since ethylene oxide is more rapidly hydrolysed to ethylene glycol in acid solution, the experiment was repeated using disodium phosphate as substrate. The mortality produced was, however, slightly lower than that obtained with the acid phosphate. Ethylene chlorhydrin was also employed as it was thought that it might hydrolyse during the drying, producing ethylene glycol and hydrochloric acid gas, but the mortality produced was actually lower than that given by ethylene glycol alone. Chloroform is often used as a preservative, but according to Bunyea[20] its vapour has little germicidal action: it was found to be ineffective when solutions containing it were spray-dried.

Although sodium hypochlorite and formaldehyde produced sterile powders, the utility of these is governed to some extent by the concentration of bactericide that they contain. Estimates of this concentration were therefore made. The available chlorine in the powder prepared from a 5 per cent. solution of sodium acid phosphate containing 1 per cent. of Milton was estimated by dissolving a weighed quantity of the powder in water, adding acetic acid and potassium iodide and comparing the colour with that of standard dilutions of Milton treated similarly, using a photoelectric absorptiometer for the estimation. No detectable amount of chlorine was found in the powder. Since the hypochlorite might be expected to be less stable in acid than in alkaline solution the experiment was repeated using disodium phosphate as the substrate, but again no detectable amount of chlorine was found. The formaldehyde in the powder was estimated colorimetrically by means of chromotropic acid and, using an initial concentration of 0·05 per cent. of formalin, the amount found in the powder was less than 0·01 per cent.

Discussion

In considering the utilisation of spray-drying as a sterilisation process due regard must be paid to the mechanisms involved in both the drying and the disinfection processes It appears probable that the solutions, on leaving the jet of the dryer, are evaporated to dryness very rapidly, in the space of a few seconds[1]. If, therefore, it be assumed that non-volatile substances can exert a bactericidal action only in solution, it is clear that these must have a very rapid action if they are to be used as sterilising agents in the spray-drier. On the other hand, during this period of drying, the solution containing the bactericide is subjected to an increasing degree of concentration which will increase its lethal activity and at the same time bring the bactericide into close contact with the suspended organisms. This enhanced activity, however, will largely be governed by the solubility and concentration exponent of the disinfectant, both of which vary with temperature. The lethal action of substances having a large value for the concentration exponent (' n '), will increase rapidly with increase in concentration, but a limit is set to the latter by the solubility of the substance at the temperature reached in the experiments. For most bactericidal substances the solubility increases with rise in temperature. The value of ' n,' on the other hand, may increase or decrease according to the substance used. Thus the values of 'n' for phenol have been shown by Tilley[21] to decrease with increasing temperature, while those for resorcinol increase. The solubility of resorcinol is also much greater than that of phenol and one might expect, therefore, that resorcinol would prove more effective than phenol in the spray-drier. The results given in Table II show that this was the case. The effect of chlorocresol was negligible and phenylmercuric nitrate, although a more efficient bactericide at lower concentrations than phenol, proved of little value; probably because the former compounds are not very soluble in water. While, however, the relative efficiencies of the phenols were in accordance with expectation, the mortalities produced were all low, except in the experiments where phenol was used with a salt substrate. Using phenol with sodium chloride, a mortality of 61·6 per cent. was obtained, which is approximately equal to the sum of the mortalities produced by these substances separately. A mortality of the same order, was, however, produced using phenol with sodium acid phosphate, a salt which itself has no lethal action. The enhancement of the activity of the phenol might, in the latter case, be due to the acidity of the substrate but the effect of the salts may also be to lower the solubility of the phenol in the aqueous vehicle and to alter the partition of the phenol between that and the lipoid constituents of the cell-wall.

Neither the anionic nor the non-ionic surface-active agents exhibited any significant lethal activity during spray-drying and the cationic compound, cetyltrimethylammonium bromide, produced only a low mortality when dried on peptone or lactose. As cationic surface-active agents are more active in alkaline medium than in acid medium[7] attempts were made to assess this effect by spray-drying the spores with cetyltrimethylammonium bromide using sodium acid phosphate and disodium phosphate as substrates. In each case, however, the activity of the quaternary compound was greatly increased, mortalities of the order of 88 per cent. being obtained, and no significant difference can be attributed to the effect of *p*H. The apparent increase in activity in the phosphate solutions may be due to "salting out" of the detergent or to the protective action of peptone and lactose. It does not appear from the results given in Table IV that the surface-active agents have markedly increased the activity of the phenols, though some increases are observable. Cetyltrimethylammonium bromide gave a diffusible precipitate with chlorocresol and peptone, which may account for the inactivation of the chlorocresol. Polyoxyethylene stearate, on the other hand, gave no precipitate with phenol but nullified the lethal action not only of this but also of the sodium chloride.

Besides the influence of concentration of bactericide on disinfection during spray-drying, the substrate on which the organisms are dried may also affect the process. The rapid evaporation of the solvent concentrates not only the molecules of the bactericide around the organisms but also many more molecules of the substrate. Substrates which do not react chemically with the bactericide at low concentrations may more readily do so when the concentration is increased. Substrates such as colloids, which show surface activity, may adsorb the bactericide if the substrate is thrown out of solution before completion of drying, and the effective number of molecules of bactericide which can be adsorbed by the bacteria themselves may be too low to have any significant lethal effect. It would appear, therefore, that the possibility of obtaining a sterile powder by spray-drying in the presence of a non-volatile bactericide is not great if colloidal matter is also present, though it is difficult to assess the importance of this effect from the experimental evidence because all the substrates used may have had some influence on the course of the disinfection.

Apart from its effect on bactericides present the substrate may have a direct lethal action on the organisms as the concentration rises, and this may be observed in the results given in Table I of spray-drying the spores using various salts as substrates. Topley and Wilson[22] state that it is doubtful whether salts, except in high concentration, affect bacteria by virtue of their osmotic pressure, although Knaysi[23] demonstrated plasmolysis induced by a 25 per cent. sodium chloride. It does not appear, however, that the results obtained during spray-drying could be attributed to the effects of osmotic pressure alone, for although the initial solutions were approximately iso-osmotic the mortalities produced varied greatly and the phosphate showed no lethal effect at all A limiting factor would, of course, be the solubilities of the salts in water, but when these are compared with the observed mortalities no degree of correlation can be traced. It is not surprising to find this variation in the action of salts, for many workers[5] have noted it when working with solutions. The very great difference between the mortalities produced by the phosphates and the other salts is, however, somewhat unexpected. While a 0·85 per cent. solution of sodium chloride was shown by Flexner[24] to cause disintegration of the meningococcus, *B. subtilis* was shown by Fischer[25] to grow well in an infusion containing 9 per cent. of sodium chloride or 11 per cent. of potassium chloride, and it does not appear, therefore, that the halides are particularly lethal to this organism. A mixture of the phosphate was, in fact, found to have a greater inhibitory effect on the growth of *Bact. coli* than sodium chloride[26], yet when spray-dried. the halides killed half the spores of *B. subtilis* while the phosphates produced no apparent mortality. Whether this is associated with the special position which phosphates occupy in relation to bacterial metabolism is a matter which requires further investigation.

Volatile bactericides may act on organisms either in solution or in the gaseous state. Many of the substances which are actively bactericidal in mists or smokes probably act in solution and Puck[13] has shown that the very high efficiency of the glycols as aerial disinfectants is due to the mobility of the gaseous molecules which collide with the bacteria-carrying particles and dissolve in the condensed layer of water which those particles bear, quickly building up a lethal concentration of the glycol. The conditions necessary for this process cannot, however, be realised in the spray-drier, for, once dried, the bacteria-carrying particles are carried forward in an atmosphere the relative humidity of which is too low to permit of condensation. It would appear, therefore, that the glycols exert their effect only during the period when the solution is being evaporated to dryness. This is confirmed by the comparatively low mortalities recorded in Table V and by the agreement of these results with the relative activities of the glycols in solution. Thus, although triethylene glycol has been shown to be a much more efficient aerial disinfectant than propylene glycol[14] it is less active in solution than the latter. Robertson[21] has ascribed the superiority of triethylene glycol to its lower aerial saturation value which causes it to condense more readily on to the bacteria-carrying particles but it is clear from the experimental results that no such effect of condensation could be demonstrated. Since, also, lactic acid shows a similar optimum humidity requirement to that of the glycols it seems probable that it acts in the same way and that its activity during spray-drying is confined to the actual period of drying. This is confirmed by a comparison of the phosphate contents of the original and reconstituted solutions, estimated gravimetrically, with the figures obtained by titrating them with alkali in the presence of excess sodium chloride. This shows that no appreciable amount of the lactic acid was vaporised and that therefore the high efficiency of the lactic acid must be due to the rapidity of its action.

The results obtained with sodium hypochlorite and formaldehyde, as shown in Table VI, are very different from the above for extremely low concentrations of these substances were sufficient to produce sterility. Masterman[16] has suggested that sodium hypochlorite acts as an aerial disinfectant owing to liberation of gaseous hypochlorous acid while Puck[13] maintains that the action of the latter is exerted in the same manner as that of the glycols. He contrasted the high efficiency of hypochlorous acid as an aerial germicide with that of chlorine which has little action and ascribed the difference to the much greater solubility of hypochlorous acid in water. Since both hypochlorous acid and formaldehyde in solution are much more lethal to bacteria than the glycols it is not unreasonable to suppose that the sterilisation produced by these substances was effected during the actual drying process but further work is proceeding to determine whether gases can exert a bactericidal action on dry bacteria, and if so to what extent.

It is evident, from the results given in Table VI, that both hypochlorite and formaldehyde could be used successfully for the production of sterile, spray-dried, powders. Certain factors however, militate against their use. They are both inactivated to some extent by protein matter: sodium hypochlorite is, in fact, completely inactivated even when a fairly heavy initial concentration is used. 0·4 per cent. of formaldehyde was sufficient to ensure sterility in the presence of peptone and only 0·02 per cent. was necessary when peptone was absent. The reactivity of these compounds and the ease with which they will combine with many substrates make them less useful than they might otherwise be. Where, however, such a consideration is inapplicable or unimportant the use of hypochlorite or formaldehyde might find application, provided that the final concentration of these substances in the powder is low enough for the purpose required.

SUMMARY

1. *B. subtilis* spores have been spray-dried, using various substrates with substances known to be bactericidal in solution. Only sodium hypochlorite and formaldehyde were found to be capable of producing sterility in the resultant powders. Phenols, synthetic detergents and glycols killed varying proportions of the spores, but none of compounds showed a high efficiency.

The reasons for this have been discussed.

2. Both sodium hypochlorite and formaldehyde were found to be largely inactivated by peptone, although in its presence formaldehyde produced sterile powders when used in an initial concentration of 0·4 per cent. In the absence of peptone an initial concentration of formaldehyde of 0·02 per cent. and of sodium hypochlorite of 0·001 per cent. was sufficient.

3. The final concentrations of sodium hypochlorite and formaldehyde in the spray-dried powders have been estimated. Using an initial concentration of 0·01 per cent. of sodium hypochlorite no detectable amount of available chlorine could be found in the powder, while an initial concentration of formaldehyde yielded a powder containing less than 0·01 per cent.

4. The effects of spray-drying the bacterial spores using various common salts as substrates have been examined. It was found that, whereas most salts exerted a lethal action on the spores, sodium phosphate and sodium acid phosphate produced no significant mortality. Further work is proceeding on the investigation of this phenomenon.

Our thanks are due to Mr. G. T. Bassil, M.Sc., for carrying out the estimation of formaldehyde in the powder.

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DISCUSSION

An abstract of the paper was read by Dr. Bullock.

MR. G. SYKES (Nottingham) asked for more details of the organism used by the authors. Some time ago they had had organisms of the *subtilis* group which were fairly highly resistant to moist heat. They could easily prepare a dry spore suspension which would stand up to dry heat at 145°C. for one hour, but not 150°C. However, this same organism in aqueous suspension died out fairly rapidly.

PROFESSOR H. BERRY (London) said that several years ago he had had a strain of *B. subtilis* which would stand up to 2½ hours' boiling, but it had died out and he could not reproduce it. It had been grown on 1 in 10 nutrient agar and maintained over calcium chloride. Although he had kept the same strain he had not been able to reproduce the heat-resisting property. It would be interesting to hear the outcome of the authors' work on aerial bactericides. Moisture content was important as it was linked with the lethal action of the bactericides. There was a difficulty in testing when formaldehyde was used as it had to be inactivated. It acted in two stages, the first reversible and the second irreversible, and for this season it was difficult to prove that the formaldehyde had really killed the organisms.

MR. W. JONES (Manchester) asked whether the authors had considered the possibility of a recovery of viability on storing. They had found this to be so with *B. subtilis* spores; after 6 months' storage there was 50 to 80 per cent. recovery. Using a modified agar medium containing starch and phenol red, recovery was demonstrated after 48 hours.

DR. K. BULLOCK, in reply, said that the organism originally came from the National Collection of Type Cultures, Warburg strain. It was not killed by dry heat at 140°C. for 1 hour but was killed at 150°C. The powders were still being stored and it was hoped to give the results of storage experiments later on. As they were concerned with the dry state they had not done much work on wet heat. Formaldehyde and hypochlorite were not seriously put forward as methods of sterilisation—they were only last resorts to see whether any substance would effect sterilisation during spray-drying. He was sure that Mr. Rawlins had stored these powders for some time before reporting them to be sterile, and they had been tested at intervals. He was not sure, however, that Mr. Rawlins had used anything to inactivate the formaldehyde.