BACTERIAL SURVIVAL IN SYSTEMS OF LOW MOISTURE CONTENT

PART IV. THE EFFECTS OF INCREASING MOISTURE CONTENT ON HEAT RESISTANCE, VIABILITY AND GROWTH OF SPORES OF *B. subtilis*

BY KENNETH BULLOCK and ALAN TALLENTIRE

From the Department of Pharmacy of the University of Manchester

Received July 2nd, 1952.

INTRODUCTION

PREVIOUS papers[1,2] in this series have shown that the spores of *B. subtilis* will remain viable in dry spray-dried powders for long periods. This is true for peptone powders, yet if such powders are dissolved in a suitable quantity of water they form an excellent medium for bacterial growth; the spores germinate, and the resultant vegetative cells rapidly multiply. Some intermediate proportion of water must be just sufficient to cause the spores to initiate the germination process. It was the purpose of this present work to determine this critical water content and to investigate the fate of the spores. Obviously they will lose their heat resistance at some point, but, the interesting question is, "will the resultant vegetative cells multiply or will they die off due to unfavourable environmental conditions?" Such questions have an intrinsic interest of their own, but also it might be possible that advantage could be taken of the facts to devise conditions under which sterility would be attained with or without the use of minimal quantities of heat or antiseptics. Methods might then be elaborated which could have direct pharmaceutical applications in the preparation of sterile powders.

Previous work related to this subject seems to have been of a qualitative rather than a quantitative nature. Thus Tompkins[3] found that the range of humidities, over which germination of certain species of mould spores is possible, varies with the temperature; the further the temperature was removed from the optimum for growth, the narrower the range of humidity over which germination occurred.

When a spore germinates a series of changes is initiated. (*a*) The spore-case cracks or is absorbed or is gelatinised and then dissolved.[4] (*b*) The spore contents enlarge, a germination tube may grow out through the envelope, or the cell may simply enlarge and divide, giving rise directly to vegetative multiplication. (*c*) The resistance to adverse environmental conditions, such as heat and harmful chemical substances falls.[4] All these changes have been used as criteria of germination. Observation of (*a*) and (*b*) involves direct microscopical examination of the individual spores. This can easily be carried out in the case of moulds where the spores are relatively large. This method was employed by Tompkins.[3] Bacterial spores are approximately one-tenth the size of mould spores and morphological changes on germination are indistinct and difficult to observe. On the other hand, as pointed out by Wynne and Foster,[5]

the heat resistance of unaltered spores compared that that of vegetative cells or germinating spores can form a very definite criterion of germination, giving reproducible results under standard conditions. This criterion has been adopted in the present work.

It is likely that the interrelationship of spore germination and moisture content will be modified by the nature of the substrate. The *B. subtilis* spores have, therefore, been examined dispersed in 4 types of powder: (1) peptone, (2) lactose, (3) equal parts of lactose and peptone and (4) kaolin. The peptone, on sufficient dilution with water, gives an excellent nutrient medium. The lactose powder does not constitute a source of organic nitrogen, while kaolin is simply an inert powder.

Materials and Methods

Except where indicated, the materials and methods used in this work were those previously described.[2] A suspension of spores of *B. subtilis* (Marburg No. 3610) prepared and stored as usual constituted the source of contamination in all experiments.

In order to obtain a sufficient bulk of spray-dried product for protracted experiments, the concentration of solids in the solutions and suspensions before spray-drying was increased to 10 per cent. and the degree of contamination was arranged to give an expected count of $2{\cdot}5 \times 10^4$ viable spores per ml. of suspension. In the case of peptone 2 batches of powder were mixed by milling. The analysis of variance of quintuplicate platings of 10 samples of the resultant powder, given in Table I, shows that the spores were evenly distributed. A powder of equal parts of peptone and lactose was prepared by drying a contaminated solution containing 5 per cent. of peptone and 5 per cent. of lactose.

TABLE I

Analysis of variance of quintuplicate platings of 10 samples of the mixed spray-dried peptone powder

Source of variance	Sum of squares	N	Mean square	Variance ratio	P
Difference between samples	1221·7	9	135·7	1·538	>0·2
Difference between individuals ..	8348·8	40	208·7		
Total	9570·5	49			

A kaolin powder was prepared by spray-drying a contaminated 10 per cent. suspension of light kaolin B.P. in distilled water. The feed bottle containing the suspension was shaken continuously throughout the drying. It has previously been shown that an even distribution of spores in a powder can be obtained by spray-drying a solution containing a suspension of spores. In the case of the kaolin suspension in spite of agitation during the drying, the spores were unevenly distributed in the resultant powder. Evenness of distribution was obtained by milling as previously found necessary with *Bact. lactis aerogenes* in peptone.[2]

Table II shows that during the drying of the 4 types of powder used, the spores did not suffer any significant mortality. The low mortality

TABLE II

PERCENTAGE OF *B. subtilis* SPORES KILLED BY SPRAY-DRYING ON THE VARIOUS SUBSTRATES

Substrate	Inlet temperature (° C.)	Mortality per cent.
Peptone, 10 per cent.	175	3·8
Peptone, 10 per cent.	175	0·0
Peptone, 5 per cent.; Lactose, 5 per cent.	80	6·4
Lactose, 10 per cent.	80	0·0
Kaolin, 10 per cent.	185	11·2

is consistent with the view that the spores in the powder were not a selected heat-resistant variety.

THE METHOD FOR THE DETERMINATION OF LOSS OF RESISTANCE TO HEAT

Loss of heat resistance was determined by placing test-tubes containing the culture, or a suitable dilution of it, in a water bath at 80° C. for 5 minutes and subsequently cooling the tubes under the cold water tap. Quintuplicate platings were performed before and after heating. In the work described below the count obtained before heating will be referred to as the total viable count, that obtained after heating as the viable spore count, while the differences between the two will be considered to represent the number of viable heat-labile organisms. A 24-hour culture containing more than 3000 viable *B. subtilis* vegetative organisms per ml. was found to be sterile after heating for 1 minute in a water bath at 80° C. Table III shows that heating at this temperature for 3½ hours had no significant effect on the viable count of *B. subtilis* spores.

The difficulty of counting vegetative cells of *B. subtilis* on account of chain formation is fully appreciated but the experiments described in this paper were terminated whenever rapid multiplication occurred, so that the viable heat-labile organisms were almost certainly spores which had lost their heat resistance in the process of germination but which had not yet given rise to typical rapidly multiplying vegetative cells.

TABLE III

EFFECT OF HEATING AT 80° C. ON THE COUNT OF A SUSPENSION OF *B. subtilis* SPORES

Time in minutes	0	30	90	120	150	180	210	240	270	300
Mean count of 5 tubes ..	162	161	171	152	153	154	151	139	133	120

CONTROL OF MOISTURE UPTAKE BY THE POWDERS

The spray-dried powders were, in the first place, further desiccated by storage over phosphorus pentoxide. For each experiment a number of approximately 0·5-g. quantities of powder were weighed accurately into sterile aluminium-capped glass tubes 7 cm. long and 2·5 cm. diameter. After removal of the caps the tubes and caps were placed in desiccators in which the relative humidity was controlled by saturated salt solutions[6] placed in the lower compartment. At intervals tubes were capped and

weighed and the contents dissolved or suspended in water (in the proportion of 9 ml. of water for 0·5 g. of dry powder, but allowing for the quantity of water known to have been taken up from the atmosphere in the desiccator). Viable spore counts and total viable counts were made on the resulting mixture.

To avoid having, at all stages, to make allowance for the increase in weight of the powders due to increasing moisture contents all viable counts were expressed as the number of organisms or spores per g. of the original phosphorus pentoxide-dried powder. For the same reason the moisture content of powders was expressed not as a percentage of the powder as it stood but as a percentage moisture uptake calculated on the original phosphorus pentoxide—dried powder. It was assumed that this original powder could be considered to be "dry." To avoid lengthy tables, graphs have been constructed from the results showing the relationships between the moisture content of the powder and the viable spore counts and total viable counts on a time basis at the various degrees of relative humidity. At high moisture contents rapid multiplication of the bacteria sometimes occurred; in such cases the graph terminates in an ascending arrow.

The humidity controls at least two factors, (*a*) the rate of moisture uptake by the powder, and (*b*) the maximum water content finally attained by the powder. Broughton and Mather[7] have reported that in desiccators containing only the saturated salt solution, the vapour and liquid phases acquire 97 per cent. equilibrium within 1 hour, but they point out that the rate of uptake of moisture by any materials exposed in the desiccator "is dependent rather upon their own (the materials') rate of attainment of equilibrium with the air than upon the rate of humidification of the latter by the salt solution." Table IV shows the saturated salt solution used and the corresponding relative humidities.

TABLE IV

RELATIVE HUMIDITY IN DESICCATORS CONTAINING SATURATED SALT SOLUTION AT 20° C.

Solid phase	Relative humidity per cent.
Disodium hydrogen phosphate, Na_2HPO_4, $12H_2O$ ..	95·0
Zinc sulphate, $ZnSO_4$, $7H_2O$	90·0
Sodium thiosulphate, $Na_2S_2O_3$, $5H_2O$	78·0
Sodium nitrite, $NaNO_2$	66·0
Potassium acetate, CH_3COOK	20·0

RESULTS

Storage of contaminated peptone powders in atmospheres of various relative humidities. The fact that the spores of *B. subtilis* will remain viable and heat-resistant, i.e., apparently unchanged in dry peptone powder for long periods of time has again been confirmed. The bulk peptone powder which formed the starting material for the various experiments described in this section has now been stored over phosphorus pentoxide for over 2 years. Viable spore counts and total viable counts have been performed

on it at various intervals and a statistical analysis of the results obtained shows that the variation in the counts does not exceed that to be expected by random sampling. The viable spore counts and total viable counts remained sensibly constant and equal at 270×10^3 per g.

When the dry peptone powder was exposed to an atmosphere of 66 per cent. relative humidity the moisture content increased in 20 days to approximately 29 per cent. and remained constant at this figure during the rest of the 8 months experimental period. Statistical analysis of the counts performed during the same period showed that the variations in viable spore counts and total viable counts did not exceed the errors of random sampling and that viable spore counts and total viable counts were the same. Thus, in peptone powders containing up to 30 per cent. of moisture the spores remain viable and heat resistant.

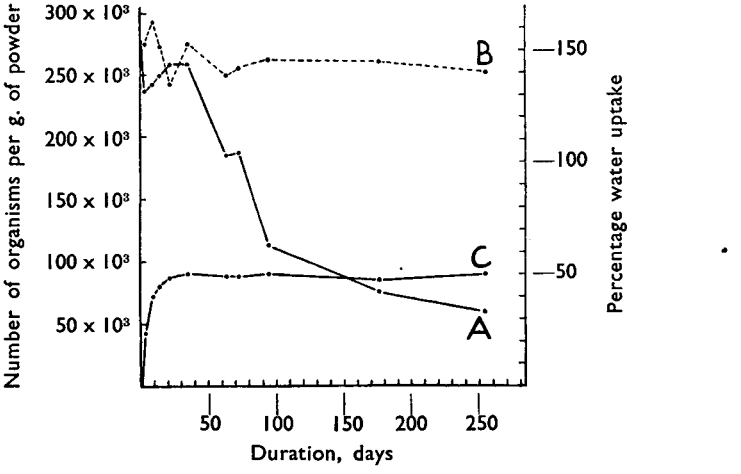

FIG. 1. Storage of infected peptone powder in an atmosphere of 78 per cent. relative humidity at 20° C.

A, Viable spore count. B, Total viable count. C, Water uptake. ● Experimental determination.

When exposed to 78 per cent. relative humidity (Fig. 1) the moisture uptake of the peptone powder was 50 per cent. in the first month and it remained constant at approximately that figure during the remainder of the 8 months. Although after 3 days (at a moisture uptake of a little over 30 per cent.) the powder became a paste, the total viable count remained constant at about 270×10^3 organisms per g. throughout the experiment. On the other hand, a little after the first month the viable spore count commenced to fall, at first rapidly, then more slowly to 60×10^3 organisms per g. after 8 months. It is clear that with a moisture uptake of 50 per cent. the majority of the spores lose their resistance to heat but remain viable.

Exposed to 90 per cent. humidity (Fig. 2) the moisture uptake of the dry peptone powder was at first rapid and, although the rate fell off subsequently, the moisture content was increasing during the whole

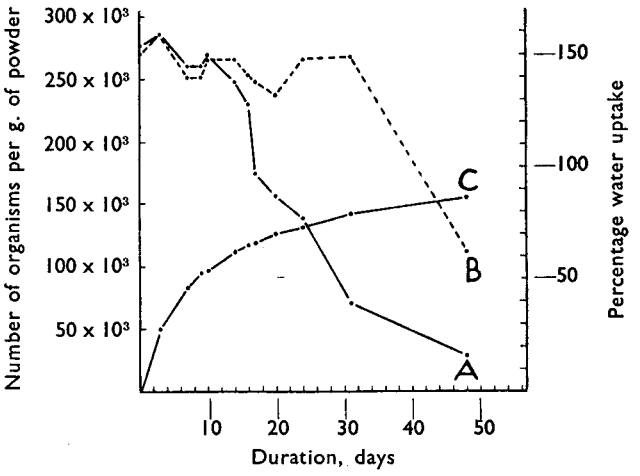

FIG. 2. Storage of infected peptone powder in an atmosphere of 90 per cent. relative humidity at 20° C.

A, Viable spore count. B, Total viable count. C, Water uptake. ● Experimental determination.

48 days of the experimental period. When the moisture uptake had risen to 50 per cent. the viable spore count began to fall while the total viable count remained constant. After 32 days at a moisture uptake of 80 per cent. with the viable spore count still decreasing the total viable count commenced a rapid fall which continued to the end of the experiment. This suggests that the spores which lose their heat resistance at a moisture uptake of 50 per cent. begin to die off when the moisture uptake has risen to 80 per cent.

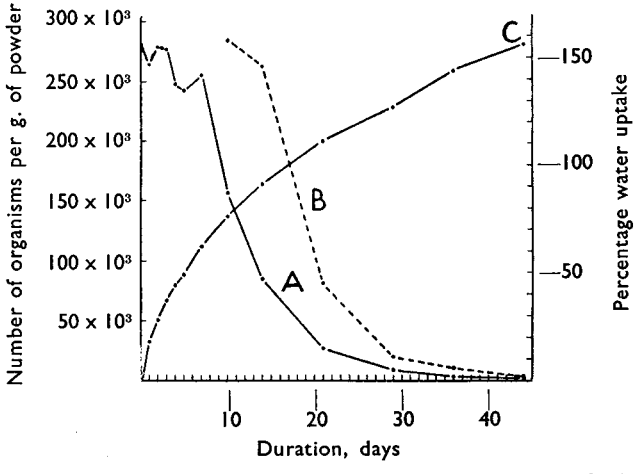

FIG. 3. Storage of infected peptone powder in an atmosphere of 100 per cent. relative humidity at 20° C.

A, Viable spore count. B, Total viable count. C, Water uptake. ● Experimental determination.

At 100 per cent. relative humidity the same picture developed a little further (Fig. 3). The moisture content rose at first rapidly then more slowly throughout the 44 days of the experiment. At 50 per cent. moisture uptake the viable spore count commenced to fall while the total viable count remained constant. At 80 per cent. moisture uptake the total viable count commenced to fall. After 44 days the viable spore count had fallen to 18×10^2 while the total viable count was approximately twice that figure although at 14 days the total viable count had been about 3 times the viable spore count.

When the 100 per cent. humidity experiment was carried out at 36° C. yet another variation occurred. Moisture uptake was rapid all the time and when it reached over 50 per cent. the viable spore count fell rapidly. No marked fall in the total viable count was however detected. This was doubtless because when the moisture uptake reached a value over 160 per cent. a rapid multiplication of the organisms commenced. The time taken for the moisture uptake to pass from just over 80 per cent. at which a fall in total viable count might have been expected to 150 per cent., the lower limit for rapid multiplication, was only about 8 days.

To confirm the above findings instead of exposing the dry peptone powder to various humidities so that the moisture uptake increased during the experiment a few experiments were carried out in which the moisture was added all at once as a weighed quantity of sterile distilled water. In this way powders with moisture uptakes of 40 per cent., 60 per cent., 80 per cent. and 100 per cent. were prepared. 2 samples at each moisture level were stored in firmly stoppered containers at room temperature. At 2 and 23 days respectively viable spore counts and total viable counts were performed on the samples. The results obtained are shown in Figures 4, 5 and 6. No loss of viability or heat resistance occurred in the spores in the powder having a 40 per cent. moisture uptake during the 23 days storage. In the samples containing more moisture, heat sensitisation without loss of viability of some of the spores occurred after 2 days. There was no fall in total viable count except, after 23 days, in the powders having 80 per cent. and 100 per cent. moisture uptakes.

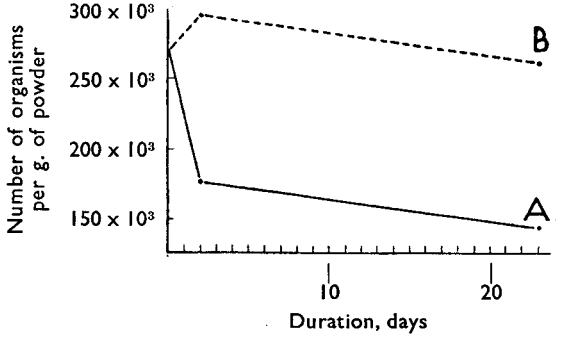

FIG. 4. Storage of infected peptone powder with an equivalent of 60 per cent. water uptake at 20° C.
A, Viable spore count. B, Total viable count. ● Experimental determination.

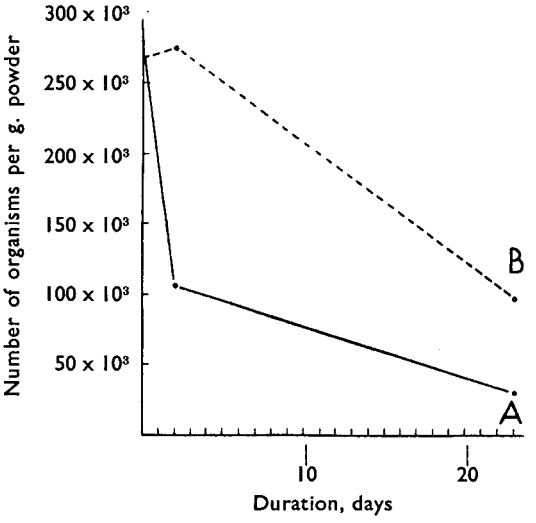

FIG. 5. Storage of infected peptone powder with an equivalent of 80 per cent. water uptake at 20° C.
A, Viable spore count. B, Total viable count. ● Experimental determination.

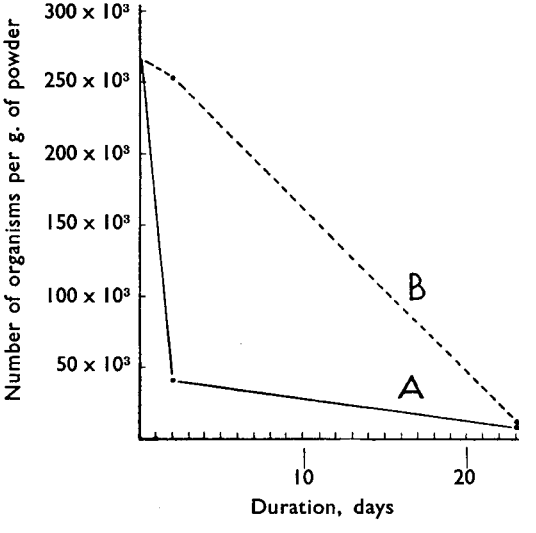

FIG. 6. Storage of infected peptone powder with an equivalent of 100 per cent. water uptake at 20° C.
A, Viable spore count. B, Total viable count. ● Experimental determination.

Storage of contaminated lactose powder in an atmosphere of 100 *per cent. relative humidity.* The results obtained with spores of *B. subtilis* in lactose powder were relatively simple (Fig. 7). The moisture content rose in about 9 hours to 9 per cent. At this point the viable spore and total viable counts began to fall rapidly for 3 or 4 days and then steadily

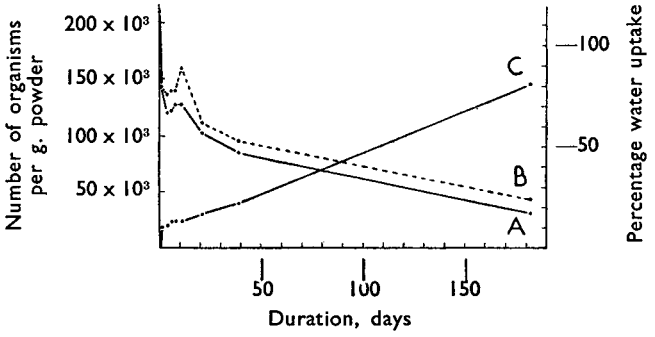

FIG. 7. Storage of infected lactose powder in an atmosphere of 100 per cent. humidity at 20° C.

A, Viable spore count. B, Total viable count. C, Water uptake. ● Experimental determination.

throughout the remainder of the 6 months period of the experiment and this, in spite of the fact that the moisture uptake increased steadily to 80 per cent. It is obvious that the organisms were losing their viability at about the same rate as the spores were losing their heat resistance.

It should be mentioned that even with high moisture uptake the lactose powders never became pastes. The powder at first becomes more coherent or "ropy" and then hard and brittle.

Storage of the contaminated lactose-peptone powder in an atmosphere of 100 per cent. relative humidity. Figure 8 shows that the results obtained with this mixed powder were intermediate between those obtained with the peptone powder and those obtained with the lactose powder. Counts remained steady at 250 × 10^3 until the moisture content reached about 35 per cent. The counts then commenced to fall, the viable spore count

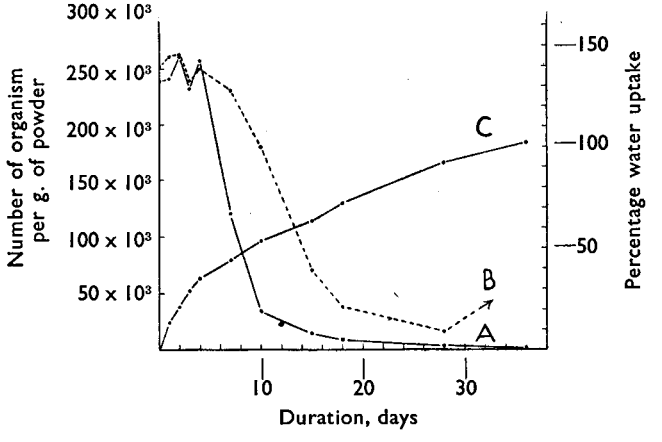

FIG. 8. Storage of infected peptone and lactose powder in an atmosphere of 100 per cent. relative humidity at 20° C.

A, Viable spore count. B, Total viable count. C, Water uptake. ● Experimental determination.

more rapidly then the total viable count, showing that the spores were losing their heat resistance at a greater rate than the organisms were losing their viability. At a moisture uptake of about 90 per cent., when the total viable count had fallen to about 20×10^3 there was a rapid multiplication of the vegetative organisms. At high moisture content this powder became first a paste and then a solution.

Storage of the contaminated kaolin powders in atmospheres of various relative humidities. The results of exposing the kaolin powders (previously dried over phosphorus pentoxide) to 20, 78 and 90 per cent. relative humidities respectively, establish the fact that under these conditions, with not more than a 2 per cent. moisture uptake, the spores remain heat resistant and viable for at least 3 months.

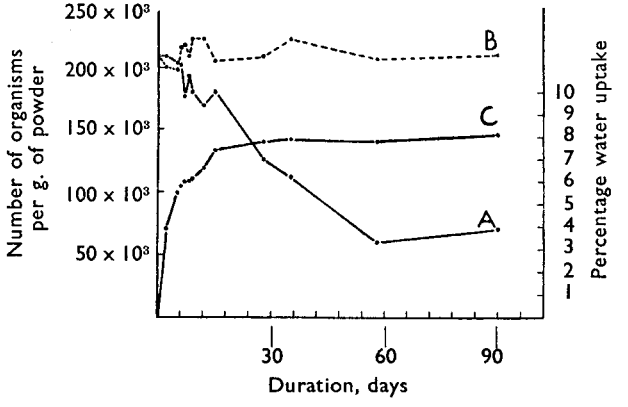

FIG. 9. Storage of infected kaolin powder in an atmosphere of 95 per cent. relative humidity at 20° C.

A, Viable spore count. B, Total viable count. C, Water uptake. ● Experimental determination.

Figure 9 (95 per cent. relative humidity) shows that, as with the peptone and lactose powders, the first observable change is a loss of resistance to heat by the spores, this time at a moisture uptake of about 6 per cent. The heat-sensitive organisms nevertheless remain viable as shown by the fact that the total viable count remained sensibly constant throughout the 3 months period. Figure 10, a record of events taking place in an atmosphere of 100 per cent. relative humidity, shows a feature found only in the case of the kaolin powders. The spores first lost their heat resistance at 6 per cent. moisture uptake, but subsequently heat resistance was regained, the viable spore count becoming, after 48 days, identical with the total viable count which had not changed throughout the experiment. There is a hint in the previous Figure 9 that recovery of heat resistance by the spores might be commencing after 60 days' exposure to 95 per cent. relative humidity.

When the exposure of the kaolin powder to an atmosphere of 100 per cent. relative humidity was repeated at 36° C. the same sequence of events occurred but more rapidly. At first the viable spore count

fell and then rose while the total viable count remained constant, indicating loss of heat resistance followed by recovery of such resistance. The spore count never regained its original value, however, because before this could occur the moisture uptake had risen to over 20 per cent., a higher value than that attained in the previous experiments and this high moisture content apparently caused death of the organisms since both viable spore counts and total viable counts began to fall and

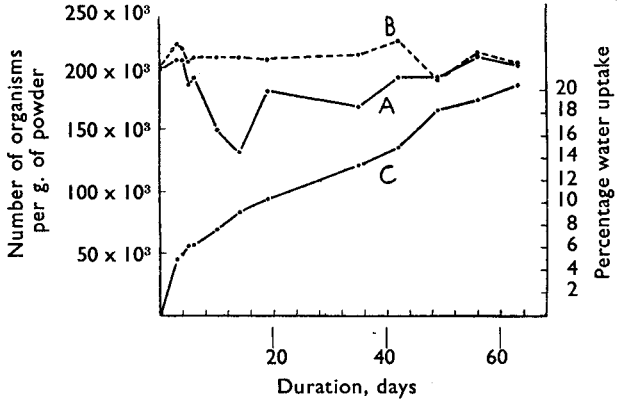

FIG. 10. Storage of infected kaolin powder in an atmosphere of 100 per cent. relative humidity at 20° C.
A, Viable spore count. B, Total viable count. C, Water uptake. ● Experimental determination.

to approach each other in value. In none of the kaolin experiments did rapid multiplication of the organisms occur. This is understandable since in such powders there is no source of either energy or organic nitrogenous food material.

DISCUSSION

When spray-dried powders containing spores of *B. subtilis* are exposed to conditions causing increasing moisture uptake 2 points may be observed irrespective of the nature of the supporting powder, (*a*) below a certain moisture uptake the spores do not appear to undergo change, since they remain for a long period of time viable and heat resistant. The value of this critical moisture uptake varies with the nature of the supporting powder, being approximately 50 per cent. for peptone, 10 per cent. for lactose, 35 per cent. for peptone and lactose and 6 per cent. for kaolin. It will be noted that for the powders containing peptone, the critical moisture uptake is considerably higher than for the lactose and kaolin powders. A possible explanation of this difference may be found in the work of Hills,[8] who has shown that, taking loss of heat resistance as the criterion of germination, inhibition of the germination process is affected by critical concentrations of certain D-amino-acids. These or other inhibitory substances may be present in the peptone and require dilution beyond their active concentration before germination can proceed.

(*b*) There is a range of moisture content in which the spores lose their heat resistance but remain viable. This range is wider in the case of the peptone powders than in the case of the lactose powders; intermediate in the case of the peptone and lactose powders. It is possible that this range is to some extent conditioned by the presence or absence of food substances, and anti-multiplication factors in the peptone. Inhibitory factors have been shown to be present in hydrolytic products of proteins,[9] but that the presence of these does not offer a full explanation is indicated by the fact that this range does occur with the inert kaolin powders. Other points dependent, however, on the nature of the supporting powder are: (*c*) above a certain moisture uptake germination of the spores and rapid multiplication of the resultant vegetative organisms occurs if suitable nutrient material is present, e.g., in the case of the peptone powders. (*d*) In the case of the kaolin powders, between that moisture uptake below which the spores are unchanged and that at which death of the spores occurs, there is a range of moisture uptake over which the spores at first lose and then regain their heat resistance. It is proposed to study this behaviour further. Unfortunately, it is not possible to ascertain the moisture content of the spores themselves in such a powder. It may be that the behaviour of the spore is relatively uninfluenced by the supporting powder, i.e., that the moisture content at which it loses its heat resistance, dies, or germinates may be always the same, the differences in the powders being explained by, for example, the greater moisture binding power of peptone compared with that of kaolin. Thus the spores in a peptone powder with an 80 per cent. moisture uptake may only contain as much moisture as those in a kaolin powder with a 2 per cent. moisture uptake. On the other hand, it is likely that multiplication and germination may be limited by the presence or absence of food materials and the presence or absence of "anti" factors as well as by the degree of availability of moisture. Perhaps the most important observation from a pharmaceutical point of view is that at a certain moisture uptake spores in a powder lose their resistance without gaining the capacity to multiply. Experiments are being undertaken with a view to determining the reaction of such spores to minimal concentrations of antiseptics.

It seems obvious that, in all powders examined, between the low moisture content at which the spores remain unchanged and the relatively high moisture content at which the spores germinate and multiplication occurs there is an intermediate zone where the spores become sensitive to adverse conditions such as heat and in fact tend to die if no further moisture uptake occurs. It will be of considerable interest to explore this zone further and to see if application can be made of it to sterilise powders for pharmaceutical use. An obvious difficulty from this point of view is the change in the physical characters of some powders to give pastes (peptone) or coherent masses (lactose).

It should be emphasised that the facts have turned out to be more complex than was anticipated. The original object of this work was to determine the one critical moisture content at which spores germinate

and to ascertain the behaviour of the spores at this point. Actually, 3 critical moisture contents have been found; there is a lower critical content below which no change occurs in the spores but above which the spores lose their heat resistance while remaining viable for up to three months at least. A second critical point has been found below which the spores lose heat resistance but remain viable and above which they tend to die off. A third upper critical point also exists, below which the spores die off and above which they give rise rapidly multiplying vegetative cells. So far as we can ascertain, the zones of loss of heat resistance with retained viability and loss of heat resistance with loss of viability have not before been reported in the literature.

It will be noticed that so far in this discussion the behaviour of the spores has been interpreted in terms of moisture uptake although it appears probable that a time factor might play some part. Thus, at first sight, the graphs of Figure 2 might be interpreted by postulating that in an atmosphere of 90 per cent. relative humidity the spores required about 15 days to lose their heat resistance and under the unfavourable conditions a further 15 days to die off. This would be an attempt to explain all the phenomena on a time basis ignoring the effects of increasing moisture uptake by the powder. It is quite probable that time factors play a part, but that they are not dominant can be seen by a comparison of the results of various experiments. It has been shown above that the spores do not lose their heat resistance for at least 3 months if the moisture content is below the critical value while curves A and B of Figures 1 and 9 show that spores which have lost their heat resistance can remain viable for more than 3 months. However, a careful examination of Figures 4, 5 and 6, where the moisture was added all at once, does clearly indicate the operation of time factors.

Certain positive conclusions of pharmaceutical interest can be drawn from this work. It is obvious that if powders containing spores are stored in a dry state there will be no germination of the spores and no increase in the degree of contamination, although the spores may retain their heat resistance and viability over long periods of time. Even if the powders are not initially dry, or if they are stored badly under conditions where moisture uptake can occur, the first change will be loss of heat resistance of the spores followed by a decrease in the degree of infection due to death of the now more sensitive spores. If exposed to conditions causing excessive moisture uptake the spores in the powders may germinate and the resultant organisms may multiply, but only if suitable nutrient materials are present. The moisture uptake necessary for this type of change would usually result in obvious changes in the physical conditions of the powder.

These conclusions have been reached using a variety of substrates, namely nitrogenous (peptone) carbohydrate (lactose), a mixture of the two and finally an inert powder (kaolin). Only one type of spore has been used, namely that of *B. subtilis* and it would of course be desirable to confirm the work using the spores of other organisms.

SUMMARY

1. When powders containing the spores of *B. subtilis* are exposed to atmospheres of increasing humidity the following sequence of events has been found to occur: (*a*) below a certain moisture content the spores remain viable and heat resistant. (*b*) Over a certain range of moisture uptake the spores lose their heat resistance while retaining their viability. On kaolin powder the heat resistance may subsequently be recovered. (*c*) At a still higher moisture content both heat resistance and viability are lost. (*d*) Above a certain moisture content in the presence of nutrient materials the spores germinate and the organisms multiply.

2. Conclusions of pharmaceutical interest are that: (*a*) Spores in a dry powder undergo no change over long periods, although they remain viable and heat resistant, there is no germination or multiplication. (*b*) If the powders are not kept in a dry condition the first change is a loss by the spores of their resistance to heat. This is followed by death of many of the organisms. Thus, far from increasing, the degree of contamination of the powder decreased. (*c*) If exposed to conditions causing excessive moisture uptake, the spores in the powders germinate and the resultant organisms may rapidly multiply in the presence of nutrient materials. The moisture uptake necessary for these changes might be expected to result in obvious changes in the physical condition of the powder.

REFERENCES

1. Bullock and Lightbown, *Quart. J. Pharm. Pharmacol.*, 1947, **20**, 312.
2. Bullock, Keep and Rawlins, *J. Pharm. Pharmacol.*, 1949, **1**, 878.
3. Tompkins, *Proc. Roy. Soc. B.*, 1929, **105**, 375.
4. Knaysi, *Bact. Rev.*, 1948, **12**, 19.
5. Wynne and Foster, *J. Bact.*, 1948, **55**, 61.
6. International Critical Tables, 1st edition, Vol. I, 67.
7. Broughton and Mather, *Amer. Dyestuff Reptr.*, 1938, **27**, 438.
8. Hills, *Biochem. J.*, 1949, **45**, 369.
9. Schumardt, Rode, Foster and Oglesby, *J. Bact.*, 1949, **57**, 1.

DISCUSSION

The paper was presented by MR. A. TALLENTIRE.

DR. K. BULLOCK (Manchester) said that he was puzzled as to why, at certain moisture levels, spores should germinate and then die off. Was that due to osmotic effects, to the fact that nutritional requirements could not diffuse to and from the organism readily, or to the presence of some anti-growth factor in the powders? Again, it was not possible at present to explain why, at a particular moisture level in the kaolin powders, the spores should first lose and then regain their resistance. The aim was to repeat the work with non-sporing organisms, but the difficulty was that non-sporing organisms died off fairly rapidly even in dry powders.

DR. K. R. CAPPER (London) said he understood that young spores were often much more resistant than older spores. There was no indication that that was related to the moisture content of the substrate.

He believed that an anti-growth factor for staphylococcus had been found in peptone, also that kaolin might contain very small amounts of nutrient material. How was dormancy related to the changes occurring in the spores? Did it occur only under dry conditions or if the spores had reduced resistance? In pharmacy the most troublesome cases were the powders which must be dissolved before injection and which could not be sterilised by heat. Very few did not provide nutrient solutions, but there would be factors such as osmosis which would affect the life of the spores which they contained.

Dr. R. M. SAVAGE (Barnet) referred to the survival of bacterial spores in surgical dressings, and said that during sterilisation there was a danger of the atmosphere not being completely saturated with steam. An extension of the investigations might throw some light on what happened at much higher temperatures than room temperature.

DR. F. WOKES (King's Langley) said he assumed that the moisture content was determined by drying in vacuo over phosphorus pentoxide. Had this been checked by the Karl Fischer method?

MR. A. MARSH (Brighton) asked whether there was any relationship between the minimum water content necessary for the germination of dry spores and the water sharing which would occur in a mixture of spores and diluent powder.

MR. D. N. GORE (Dorking) said that in the substrate there was a difference between the mobile and the total moisture, so far as the organism was concerned.

MR. P. LAWRIE (Edinburgh) said that in his experience with surgical catgut the Karl Fischer reagent gave high results due to combination of the iodine with protein.

DR. K. BULLOCK (Manchester) said that the authors had used powders dried over phosphorus pentoxide and reported the moisture uptake. That was why the phrase "moisture uptake" was used.

MR. A. TALLENTIRE, in reply, said that *Streptococcus fæcalis* was found to be the best non-sporing organism for spray-drying purposes. A culture of spores all of one age was used; consequently comparative resistance of young and old spores did not enter into the work. Anti-growth factors had been found in peptone and work might be carried out on separating them later. One or two spores remained dormant up to 12 months, and it had not been possible to get them to germinate by increasing the moisture content. The method used for the determination of moisture was the difference in weight, and moisture uptake, not moisture content, was recorded.