HEAT RESISTANCE OF BACTERIAL SPORES

ON THE HEAT RESISTANCE OF BACTERIAL SPORES.

WITH A CONSIDERATION OF THE NATURE OF THE SPORE-LIKE BODIES SEEN IN B.TUBERCULOSIS AND ALLIED FORMS.

THESIS FOR THE DEGREE OF MASTER OF SCIENCE.

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by

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INTRODUCTORY.

Every bacteriologist will agree that the proof of the spore lies in its resistance. There is abundant evidence on every hand that its resistance to heat and disinfectants is markedly superior to that of the vegetative non-spore bearing stage of the bacillus from which it is developed, or of non-spore bearing bacteria in general. The B.Anthrax spore, commonly but mistakenly regarded as "the most resistant known", is employed as the test par excellence of efficiency of disinfectants. Koch found that the bacillus of B.Anthracis is killed by an exposure to one percent phenol for two minutes but that the spores, subjected to solutions of this strength, survive from one to fifteen days[1]. The spore's greater capacity to withstand heat, particularly moist heat, is attested by general statements in all the text-books. "The temperature necessary to kill bacteria is not far above 60°C. for ten minutes, in a moist condition, where spores are present 90° to 100°C. is required, and this must sometimes be applied by the intermittent method". In using the Arnold steam sterilizer we have always assumed that the average spore would stand twenty minutes in steam at 100°. McFarland[2] states that some spore-bearers are able to withstand boiling for an hour. Sternberg

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1. Frost and McCampbell: "General Bacteriology", p. 49.
I purposely quote here from the ordinary text-book rather than from special and advanced works on the subject, in order to emphasize the general trend of opinion upon the matter of resisting qualities of spores.

2. McFarland: "Text-book upon the Pathogenic Bacteria".

notes that while the spore of B. Anthracis requires four minutes boiling to kill it, the spores of B. Alvei, Wurtzel bacillus, and B. butyricus withstand a similar treatment, as also that Globig worked with soil bacteria which resisted streaming steam for five to six hours. Madzsar[3] did not destroy the spores of B. gangraena pulpae of Agkovia by the action of steam at 100° for twenty-three minutes, and according to Wiel[4] B.mesentericus ruber will ordinarily succumb only after being subjected to steam for from one to six hours. These statements point to a recognition of specific variations in thermal resistance on the part of endospores. There is, however, a singular lack of definite data as regards the thermal resistance of endospores in general and of investigations coordinating these data regarding the resisting powers of the spores of individual species; - a search through Baumgarten's Jahresbericht for the last ten years covered by that publication (i.e. for the years 1901-1910 inclusive) reveals not a single investigation of this nature.

The sporadic, unscientific state of our knowledge in this respect is exemplified by the contradictory views brought forward in explanation of certain supposedly resistant bodies associated with Tubercle bacilli. More particularly in old cultures or old foci of tuberculosis, small round or oval bodies are found and the bacillus itself may take on a beaded form. Young rods, at first Gram negative, lose this character with age;

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3. Ctbl. f. Bakt. 29: 1901: p.745.

4. Ctbl. f. Bakt. Abt.I : 1911 : 30 :500-526.

these bodies are Gram positive. They may be found in obsolescent, encapsulated, caseous tubercles in the lung when the most careful staining fails to reveal a single tubercle bacillus. As methods of staining this organism both in tissue and in smears have been most fully elaborated, it does not seem possible that any bacillary rods present escape detection. On the other hand matter free from true bacilli but containing these resistant forms will set up typical tuberculosis when inoculated into guinea pigs. These granules grown upon a favourable medium, moreover, can be made to revert to the typical acid-proof Gram negative form of the Bacillus Tuberculosis[5]. Do these bodies then represent resistant forms - spores - of the specific organism of tuberculosis? In contradiction it is urged that they have not the full resistant power of true spores since subjection to a heat of 80° C. will render this caseous matter innocuous. But there is no conclusive evidence establishing a resistance to a temperature of 80° C. as the lower boundary of all true spore territory. Granting the known variation in the powers of resistance between species there is the possibility that the notable extremes of high resisting power may have their counterpart in those of low resistance. If it were found, on submitting the spore-bearing bacteria to a uniform heat test, that they fell into a series varying from those with spores resisting the moist temperature of boiling water, or even higher, down to those withstanding heat a few

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5. W. B. Wherry : Jour. Infect. Dis.: Vol.XIII, 1913 : p.144.

degrees higher than that fatal to bacterial bodies, then in this table we should include as true spores the beaded bodies of B. Tubercle - the Much's granules of Hodgkin's disease, and the allied forms seen in old tubercular foci, provided we find these possess higher resisting powers than do the vegetative bacilli. In addition, this scale of resistance between spores might well be a further means of differentiation or diagnosis between species.

It was with the two-fold object, first, of determining more precisely than hitherto the limits and the specific differences of heat resistance in various bacterial endospores, and secondly, to determine whether the evidence can be adduced which would allow us to regard these bodies in the tubercle bacilli as spores, that the present investigation was entered upon.

THE METHODS EMPLOYED.

Previous experiments on the heat resistance of spores shows that there are many different factors influencing the thermal death point of endospores which have to be taken into consideration. Differences in the constitution and chemical reaction of media, surface tension, age and the previous history of cultures, and particularly the nature of the heat employed, must all be taken into account in order to obtain uniform results. The disturbing influence of these factors may, however, be counteracted to a large extent by the simple expedient of subjecting all the cultures tested to an identical procedure, both as regards preliminary growth and age of growth, and as regards medium and mode of testing. Our object was not to

study these factors of variation, but on the contrary by subjecting all the various cultures to the same procedure to gain thus constants which would permit us to answer surely the main question: do different species of spore-bearing bacteria present constant differences in the susceptibility of their spores to the action of moist heat?

Preliminary observations demonstrated that surface cultures upon agar-agar media of the various forms employed showed no obvious alteration in the resistance of the spores between a week and two months. Such surface cultures were therefore employed throughout, and for each test a suspension of the spores and spore-bearing bacteria taken from an agar culture at least a week old was used. The medium of suspension was standard "Lemco" peptone bouillon rendered one percent acid to Phenolphthalein. That they might heat up rapidly, small thin-walled test tubes 0.5 mm. thick and 12 mm. in diameter, were half filled with the broth and brought to the required heat in the water bath about to be described; when the proper temperature was attained the spores were introduced by means of a platinum loop. Our early experiments had impressed us with the difficulty of maintaining a constant temperature, and more particularly with the slow arrival of the fluid within a test tube at the temperature of the surrounding fluid. For this reason it has seemed to us that the routine exposure of spores to a stated temperature for ten minutes or one quarter of an hour too frequently gave false results, since for a considerable part of the time of exposure the spores might be subjected to a

temperature below that recorded in the immediately surrounding fluid, when the temperature was taken not within the test tubes, but in the surrounding fluid. It therefore appeared that this source of error would be eliminated, and a more thorough test afforded, if the period of exposure were extended from fifteen minutes to an hour. Thus unless otherwise stated, our results represent the exposure of the spores in suspension to a given heat for a period of one hour. At the end of sixty minutes the tubes were taken out of the bath, immersed in cold water, and surface inoculations made upon Lemco peptone broth agar, the original tubes being employed now as controls to demonstrate the complete destruction - or otherwise - of all the spores.

THE WATER-BATH.

Studying the earlier literature, it is impossible not to realize that the methods employed by most observers, whether to test the thermal death-point of bacteria, or to determine the heat resistance of endospores, have been very imperfect. In the course of these observations an endeavor has been made to eliminate as far as possible the imperfections.

1. As already indicated, if a glass tube containing fluid be immersed in a water-bath, it requires many minutes before the contents of that tube attain the same temperature as that of the surrounding fluid. Therefore to determine accurately the temperature to which a suspension of spores is exposed it is essential that the thermometer be immersed not in the surrounding fluid, but actually within the suspension itself, in other

words, for accurate work the amount of a suspension of spores employed must be sufficiently large to permit the placing in it of a thermometer or, what comes to the same thing, a control test tube must be employed in which the thermometer is immersed. This must be of same size and contain same amount of fluid as the test tubes that are employed to hold the suspensions of spores.

2. The amount of loss of heat from the free surface of a water-bath is much greater than, we think, is ordinarily imagined. At temperatures between 60° C. and 100° C. it was found that this loss was so great that employing a large water-bath of two jackets we repeatedly found it impossible in the dry winter air of Montreal to maintain the water in the inner receptacle within three or four degrees of that in the outer. When using a very large surface, as, for example, that of the routine "serum inspissator", with which we made our first experiments, the water in the outer compartment might be boiling while that in the inner could not be raised above 94° or 95° C. This great loss of heat is obviated by covering the various compartments of the water-bath. By this means we were eventually able to bring the temperature of our different compartments to within a degree of difference within five to ten minutes.

3. It seemed to us - although here we may be introducing unnecessary refinement - that the temperature in our control test-tube could be preserved at a more even level if, instead of the ordinary two compartments, we suspended in the ordinary large water-bath (about a foot across, outside measurement)

a beeker filled with water to contain the tubes which were being tested. This method, it is true, by bringing the surface exposed during the various manipulations to a minimum, reduced the amount of evaporation and loss of heat. In this way we found that we could without difficulty keep the contents of our test-tubes for sixty minutes at a temperature which with occasional and by no means constant oversight varied within but two degrees during the experiment.

4. In his observations upon the thermal death point of tubercle bacilli present in milk, Theobald Smith[6] has explained the occasional survival of tubercle bacilli after an exposure as long as sixty-five minutes to a temperature of 60° C. (whereas in bouillon and distilled water at most fifteen to twenty minutes is necessary to kill all bacilli at this temperature) by the formation of a surface pellicle over milk heated in the air. In this, he states, the bacilli are carried by fat globules which shield them from the effects of the heat. Our observations suggest to us that here Theobald Smith has not afforded the whole explanation. It is not the mere formation of the pellicle that preserves the bacilli, but in addition this pellicle is the site of evaporation and cooling so that its temperature in consequence of evaporation is distinctly lower than that of the mass of the underlying fluid. Milk suspensions of the bacilli in sealed pipettes were killed in the usual time. As has been pointed out, this surface evaporation and rapid loss of heat is easily prevented in the water-bath by closing the various compartments above. It has seemed to us that a

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6. Jour. Exper. Med., Vol.IV : 1899 : p.217.

similar arrest of evaporation and so of surface cooling is to be obtained by tightly plugging the test-tubes with cotton wool. To make quite sure, however, that out results or some of them, have not been due to this cooling of the surface film, we have made tests in which four test-tubes containing an equal amount of Anthrax spores have been placed for sixty minutes in the water-bath at a temperature of 85°C. Two of the test-tubes had poured into them a covering layer of albolene, the other two being employed as controls. At the conclusion of the heating, agar plates were made from each of the four tubes. These results were as follows:-

The test was repeated three times, each time with greater accuracy, and each time, strange as it may seem, the average number of colonies on the plates from the broths protected by albolene was greater than that of the other plates. Clearly the protective effects of a surface tension layer has been overcome.*

As the result of these various preliminary tests, we employed for these observations the water-bath represented in the accompanying diagram. The compartments A and B represent the compartments of the ordinary water-bath, C represents a glass beaker 4 inches in diameter, fitting into and suspended by a circular opening in the copper disc D made of 0.1 inch copper. Covering C. is another disc, E, of the same metal provided with a dozen openings, each nine-sixteenth of an inch in diameter, permitting thus the passage through them of test

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* A possible explanation of this result is that given by Theobald Smith, namely, that oily matter in the fluid forms a protective layer about those spores which came into immediate contact with the layer of albolene.

tubes to half an inch in diameter. The test-tubes, it will be seen, are immersed so that the level of the contained fluid is well below the level of the water and that the air is also heated.

Since elaborating this apparatus we have found that Rosenau[7] has employed a very similar apparatus. He likewise has closed in the upper surface of his water-bath, and has determined the thermal death point by placing the thermometer within the test-tubes in which are the suspensions. On the other hand he employed two, not three compartments, and says nothing about closing the test-tubes so as to reduce evaporation and surface cooling.

What is quite the most accurate and delicate instrument yet devised for testing the heat resistance of spores is the apparatus of C. Balfour Stewart[8], in which the outer jacket contains benzol boiling at 80°C.; the inner closed chamber, whether it contains air or water, if worked properly can thus maintain a constant temperature of 80°C. This instrument is admirably adapted for testing the resistance of spores to what may be termed the critical temperature of 80°C. over periods of different length - five minutes, ten minutes, an hour, etc. For this research in which it is desired to determine the resistance of endospores subjected for a constant time to different temperatures, the apparatus cannot be employed.

A point in our procedure that is sure to be criticised is the employment of peptone broth for our suspensions rather

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7. U.S. Treasury Dept.,Hygiene Lab. Bulletin No.42, Jan.1908.

8. Thompson Yates Laboratory Reports,Vol.III,Pt.1, 1900, p.38.

than distilled water. It is generally accepted that the thermal death point of bacteria varies somewhat according to the medium on which they are suspended, and thus as not all laboratories employ the same method of making broth, should they therefore endeavor to repeat these observations, they might obtain results not wholly consistent with ours. In the course of these observations we found as a matter of fact that the employment of melted peptone-broth-agar as the medium of suspension did very materially raise the death point of Anthrax spores. With so thin a solution as ordinary peptone broth apparently little change is brought about in this respect. Thus Theobald Smith found no difference in the thermal death point of tubercle bacilli suspended in distilled water and peptone broth respectively, and tubercle bacilli are more susceptible organisms than are endospores. As already stated we selected this broth in order that we might thus gain additional controls, and this advantage appeared to us to outweigh any possible disadvantages.*

THE CULTURES EMPLOYED.

Using this apparatus we have tested twenty-six different cultures of sporulating bacteria. Some half a dozen others either died out or became contaminated during the course of experiments, and as the records are not perfect we have

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*Anthrax spores were killed in an hour at 90°C. when suspended in broth, in agar suspensions as noted later, some few spores survive this temperature.

not included them. For the majority we are indebted to Professor Gruner, late Pathologist to the Royal Victoria Hospital, who has made extensive study of the B.subtilis group[9]. Unfortunately Dr. Gruner left Montreal in March, leaving behind no notes as to the species differentiation of many of these forms. Many were merely designated by number. We learn from him that these were members of his collection not necessarily members of the subtilis group, which for one reason or another he was reserving for future study. Time has forbidden that we should make a full study of the differential characters of these forms, while further we have felt some little delicacy in labelling them prematurely. The other portion of our collection we owe to the courtesy of Dr. Winslow who has afforded them from the well-known collection maintained at the American Museum of Natural History, New York. I would here express my very sincere thanks to Dr. Gruner and Dr. Winslow for their courtesy.

RESULTS.

For the present we would state the results obtained with bacilli possessing true endospores, leaving for separate consideration the further discussion regarding the significance of the granules in the tubercle bacillus and allied forms. The various species grown in pure culture and the suspensions made as already described, were subjected for sixty minutes to temperatures ranging in differences of five degrees from 55°C. to one hundred. Or more accurately, most observations

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9. Gruner and Fraser: Observations on the B.Mesentericus and allied organisms. Jour.Infect.Dis.,Vol.X, :1912 : p.210.

were made first at the temperature of 80°C. so frequently employed in tests upon spores, and from here onwards successive estimations were made of five degrees difference, both above and below this temperature. The accompanying chart affords more eloquently than any words the results obtained.

In studying the chart and the results therein indicated, it must be called to mind that previous workers have most often afforded the results of fifteen to twenty minute exposure at different temperatures. Many forms which will survive exposure for fifteen minutes at 80°C. are killed by exposure for half an hour, and yet more by exposure for an hour. The test is distinctly severe. Secondly, whereas in most of the observations there has not been a variation of more than a degree on either side of the temperature indicated, in some there has been a variation of two degrees on either side, thus 80°C. indicates a temperature which during the hour may have varied between 78 and 82°. With rare exceptions the results have been harmonious, occasionaly thus there have been forms which have given no cultures after exposure to 85°, whereas a few colonies have survived 90°C. In all such cases careful retests have been made so that we believe that this chart affords an accurate table of the thermal resistance of the forms employed. The positive sign indicates that the strain in question and its spores were not wholly killed off by the given temperature, and that cultures were obtained upon inoculation.

CHART OF THE HEAT RESISTANCE OF ENDOSPORES.

	Centigrade. 70°	75°	80°	85°	90°	95°	100°	Source.
"533" (1)	+	+	+	+	+	+	+	Royal Vic.Hosp.
"633"	+	+	+	+	+	+	+	Dr.Gruner-large surface colony
B.gastrophilus	+	+	+	+	+	+	-	do.
"533" (2)	+	+	+	+	+	-	-	do. -lobed colony
"Spore 8"	+	+	+	+	+	-	-	Contamination of B.ochraceus
"Spore 7"	+	+	+	+	+	-	-	Contamination possibly B.subtilis.
"Spore 1"	+	+	+	+	+	-	-	Aerobe isolated from Blackleg virus.
B.petasites	+	+	+	+	+	-	-	Dr. Gruner
B.ochraceus	+	+	+	+	+	-	-	Dr. Gruner
B.subtilis	+	+	+	+	+	-	-	Dr. Gruner
B.megatherium	+	+	+	+	+	-	-	Dr. Gruner
B.liodermos	+	+	+	+	+	-	-	Amer.Mus.of Nat. Hist.(Dr.Winslow
B.plicatus	+	+	+	+	+	-	-	do.
B.subtilis	+	+	+	+	-	-	-	do.
B.mycoides	+	+	+	+	-	-	-	do.
B.megatherium	+	+	+	+	-	-	-	do.
B.vulgatus	+	+	+	+	-	-	-	do.
B.gummosus	+	+	+	+	-	-	-	Dr. Gruner
"634"	+	+	+	+	-	-	-	do.-reticulated growth.
"Spore 19"	+	+	+	+	-	-	-	do.-wrinkled brown growth
B.anthracis	+	+	+	+	-	-	-	Laboratory stock culture.
B.mesentericus	+	+	+	-	-	-	-	Dr.Winslow
B.anthracoides	+	+	+	-	-	-	-	do.
"623"	+	+	+	-	-	-	-	Dr. Gruner
B.cereus	+	+	-	-	-	-	-	Dr. Winslow

DISCUSSION OF CHART.

Nevertheless attention must be called to certain very important points that have presented themselves during the course of this investigation. These I will take up in detail.

1. It is obvious that all endospores do not possess the same resisting power to heat.

2. Very few spore-bearing species possess spores which are capable of surviving a moist heat of 100°C. for sixty minutes. Only two of the series possess this power.

3. B.Anthracis, which is so often spoken of as the type resistant sporulating organism, will only survive a temperature of 85°C. for one hour, thus coming relatively low down in the list. The strain employed by us was an old stock laboratory strain which had been in the Pathological Laboratory of McGill for many years.

4. At the other extremity of the scale is a culture of B.cereus obtained from Dr. Winslow which failed to survive exposure to 75°C. for one hour. This form possesses what otherwise were typical endospores.

5. So far as it is safe to draw any conclusions from this restricted series, an hour's exposure to a temperature of 70°, rather than 80°C., affords the test for the existence of endospores.

6. While this is the case, it has been most noticeable that the temperatures here given are not the temperatures resisted by all the spores of a given culture; on the contrary the majority of the spores are killed by the standard exposure to temperatures

10 - 20 degrees below those here indicated. Or in other words, the highest positive sign in the chart represents the upper limit of heat resistance possessed by a minority of the spores of any given culture in our series: it represents the maximal resistant power of the spores exhibited by some only of the endospores present in a given culture of a given strain of a bacterial species. The heat resistance of spores is thus very far from being a constant quantity, even if possibly in a given species different cultures have this in common, that they will afford a certain number of spores having the same maximal thermal death point.

We do not believe that this is generally recognized. The following experiment is one of a large number which we have undertaken. Two sets of agar tubes were melted by boiling, then the first was placed in the water-bath and allowed to come to 90°C., the second was cooled in water to about 40°C. The culture of B.Anthracis and six cultures of other bacteria which had resisted a temperature of 90°C. for one hour in broth were selected. A uniform loopful of these spores in each case was transferred to a tube containing about one centimetre of broth, and made into a uniform emulsion, and without delay a standard loopful of each emulsion was transferred to the melted agar tubes of the first series, and of the second series respectively. That at 40°C. was immediately poured upon a Petri dish for use as a control. The 90°C. agar was maintained at this heat for one hour, and then also plated. After twenty-four hours the number of colonies on the two plates were compared. The heated showed an enormous reduction in the number of colonies that developed.

In fact the vast majority of the spores are destroyed with this length of exposure at this temperature; there are only rare survivors. Thus to give an example, the plates afforded the following results:-

Culture "No.1" Control, 150,000 Subjected to 90°C. 10 colonies.

B. Anthracis Control, 37,500 Subjected to 90°C. 2 colonies.

Here, regarding the anthrax bacillus, it must be recorded that we have found the endospores to resist a temperature five degrees higher when heated in agar broth than in plain peptone broth.

7. Different strains of organisms of the same species are liable to exhibit different resisting powers. This fact has been noted by previous observers. Thus more particularly A. Schmidt[10] has called attention to the varying resisting powers exhibited by the spores of B.Chauvei. He too has observed that it is only certain of the spores that possess stfong resisting powers, the majority being rapidly destroyed. He found that the spores obtained from cultures were less resistant than those taken directly from the flesh of animals which had died from Quarter Evil. Several observers, among them Wiel[11], Kokubo[12], and Pfeiler[13], have called attention to the varying resistance of the spores in different strains of the Anthrax bacillus. In our own series it will be observed that we studied two strains of B.Megatherium and of B.Subtilis respectively, obtained from well accredited sources, and that the two strains show a difference of a few degrees in their maximal thermal resistance.

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10. Inaug.Diss. Bern - Strassburg, 1906.
11. Ctbl. f. Bakt. Abt.I. 30 : 1911, p.500
12. Ibid. Vol.34: 1903, 725.
13. Ztsch.f.Inf.u.Paras. Krankh.u.Hyg. d.Hausthiere 1:1906:124.

Taking all these data into consideration, it becomes evident (a) that in any one culture the spores present exhibit marked difference in the degree of their resistance to moist heat, and (b) that in any one species different strains vary in their maximal heat resistance. It is very obvious therefore that one of the questions proposed in our introductory paragraphs must be answered in the negative:- the heat resistance of the endospores of any given spore-bearing bacterial species cannot safely be employed as a means of species differentiation.

ON THE HEAT RESISTANCE AND THE SIGNIFICANCE OF THE GRANULES IN TUBERCLE AND ALLIED BACILLI.

I now come to the second part of this investigation, namely to the study of the relationship of the bodies seen in tubercle bacilli to endospores proper, as determined by their heat resistance. For the purpose of this research I am greatly indebted to Dr. W. B. Wherry of Cincinnati for providing me with the culture of a strain of B.tuberculosis, which exhibits the spore-like bodies to an extent rarely observed. The culture in question was brought to America from Koch's laboratory in Berlin by Prof. V. C. Vaughan of Ann Arbor in 1888, and has been cultivated outside the body ever since, until now it has attained an extreme degree of saphrophytism, growing easily on the ordinary media of the laboratory, and forming colonies which are visible in the course of three days. Dr. Wherry has described and figured this organism,*and we can wholly confirm his general description.

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*Ctbl. f. Bakt., Erste Abt., Originale 70:1913:p.115.

See also Jour. Infect. Dis. XIII:1913:p.144.

Nocard and Roux[1] observed the spore-like bodies in old cultures of the tubercle bacillus particularly when stained by Ehrlich's method. Metchnikoff[2] has described them in tuberculous sputum, as also in the anterior chamber of eye of a rabbit dead of tuberculosis. Klein[3] found them in glycerine-agar and broth cultures, and they have been described and pictured by Coppen Jones[4]. These spore-like bodies are found frequently in preparations from old pulmonary cavities as rounded bodies of greater diameter than the tubercle bacilli; from one to three or more may occur in a single rod. They take up the dye strongly, and still retain it when the rest of the bacillary body is decolorized by nitric acid. These bodies form abundantly in this strain obtained from Dr. Wherry, and we have found that with age they become Gram positive. I herewith reproduce a figure of these bodies afforded by Dr. Wherry (plate II) drawn after vital staining with the Casselman Company's new methylene blue. It will be seen that these bodies have a distinctly spore-like appearance although they differ from spores proper in that several may be present in one single bacillary rod.

Within the last few months bacilli presenting the same "diphtheroid" appearance have been isolated by Bunting, Rosenau and others* from the enlarged lymph glands in the

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1. Ann. de Pasteur : I. : 1887 : p.19.
2. Virchow's Arch., Vol. 113 : 1888 : p.63.
3. Ctbl. f. Bakt., Bd. 7 : 1890 : p.793.
4. Ctbl. f. Bakt., Abt.I : Vol.XVII : 1895 : p.1.

*I am indebted to Prof. Adami for this description of the organism associated with Hodgkin's disease.

remarkable condition known as Hodgkin's disease, or lymphogranulomatosis. Some years ago Fränkel and Much studying material from this disease, discovered and figured in the enlarged glands granules and occasional beaded bacilli which now are known as "Much's granules", closely resembling the granules and beaded forms of the tubercle bacillus, and at last year's meeting of the German Pathological Society many of the speakers supported the view that these represented an attenuated form of the B.tuberculosis. The organism isolated by Bunting has, however, such different growth characters that at the present time we must regard it as belonging to a different species. Through the great kindness of Prof. Rhea, I have been enabled to study and employ a culture of these diphtheroid bacilli isolated by him from a case of Hodgkin's disease that occurred at the Montreal General Hospital. (See Plate 4).

This very fact that the bodies under debate are apt to be multiple in the course of a single bacillary rod, the further fact that both the tubercle bacillus and the B.diphtheroiae (which also possesses metachromatic beading in many of its strains) show, under certain conditions, well marked branching, has led to a general consensus of opinion of late years that these two species are to be placed among the so-called "higher bacteria" close to the group of Streptothrices, of which the Actinomyces hominis, the organism of actinomycosis or "lumpy jaw" is the best known example. Now these streptothrices while very minute, have all the characters of the lowest moulds or so-called

hyphomycetes. They form a densely felted branching mycelium, and the peripheral threads are liable to break up into a succession of beaded gonidia; at other times the fine hyphae break up into bacillary forms. It is becoming thus not unusual to compare the beaded forms in the tubercle bacillus with the more typical gonidia-like bodies of the streptothrices. (See Plate 3)

THE THERMAL DEATH POINT OF THE GONIDIA OF MOULDS.

It seemed thus interesting to observe whether the asexual fructifications, spores, or gonidia, of the commoner moulds possess the same heat-resisting properties as do the endospores of bacteria. It is not a little remarkable that scarcely any attention seems to have been given this question. I have found only one reference to the matter in the course of my reading, and that merely the vague passing statement that the thermal resistance of the spores of forms like Mucor and Penicillium is distinctly less than that of bacterial endospores. It seemed worth while, therefore, to study this matter in its broader aspect to observe what is the resisting power of the gonidia of the lower moulds, and to compare this with the resisting power of the presumed spores of the tubercle bacillus.

Having obtained luxuriant growths of two fungi, Penicillium glaucum and Mucor ? Sp.* on bread exposed to the air of the laboratory, we subjected them under the same conditions to the test of heating in broth. After the required period of heating, the broth was cooled and poured upon sterile bread in

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*This was a mucor having the general characters of Mucor mucedo, though the Sporangia were smaller than is usual with this species.

test-tubes. The first experiment of that/heating for sixty minutes at 100°C., then, 60°C. Following these was a series decreasing both in the degree of heat, and in the length of the time of exposure, until at length it was found that the gonidia would not germinate after heating for ten minutes at 40°C. This is a confirmation of the preceding statement that they are distinctly less resistant to heat than are endospores. Evidently with a very moderate heat the spore case of these large gonidia becomes ruptured when they are immersed in fluid.

THE THERMAL DEATH POINT OF THE PRESUMED SPORES OF B. TUBERCULOSIS.

The method used for testing the tubercle bodies was varied to the extent that all growths of this bacillus were planted upon either Dorset's egg medium or upon glycerine agar, which media are known to be much more satisfactory for this organism than plain agar-agar; the Diphtheroid bacillus of Hodgkin's disease was transferred to hydrocele agar. As showed years ago by Prudden and Hodenpyl[10], the intravenous inoculation of tubercle bacilli killed by heat, results in the production of characteristic tubercles around the clumps of the dead bacilli where they become arrested in the capillaries. While these tubercle-like growths eventually become absorbed and disappear, and ~~xxxxxxx~~ no progressive infection is set up, these "pseudo-tubercles" nevertheless by their presence delay a sure diagnosis regarding the vitality of inoculated bacilli. It ~~was~~ therefore inadvisable to employ animal inoculation as

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10. New York Med. Jour., June 6th & 20th, 1891.

the test, in researches upon the thermal death point of tubercle bacilli. The culture method becomes thus the most convenient and at the same time the only indisputable way of ascertaining the resistance of these bacilli to heat. The following table represents the results of tests of the same character as those undertaken with the endospores and the gonidia.

	55°C. 15 min.	55°C. 30 min.	58°C. 60 min.	65°C. 10 min.	70°C. 10 min.
B. tuberculosis (typus humanus) Laboratory culture	+	-	-	-	-
B. tuberculosis (Wherry's strain)	+	-	-	-	-
Diphtheroid Bacillus from Hodgkin's Disease (Dr. Rhea)	+	-	-	-	-

It is clear from the above observations that these bodies seen in tubercle bacilli, as also in the diphtheroid bacilli obtained from Hodgkin's disease are in the first place very much less resistant to moist heat than are any of the obvious endospores studied in this series, and in the second place, that their resistance is not greater than that of ordinary non-granular or non-beaded tubercle bacilli. Wherry has already come to the same conclusion with regard to strains studied by him.* The same has been the experience of most observers both as regards the resistance to heat, and the resistance to chemicals.

* "When suspended, 0.85 percent NaCl. solution these cultures were not killed at 15 minutes nor 30 minutes at 55°C., but were killed by heating to 60°C. for 15 minutes." (Jour. Infect. Diseases, Vol.XIII : 1913 : 114.

Thus to take one who is perhaps the most careful and exact bacteriologist of our time, Theobald Smith[14] finds that suspensions of the caseous matter from bovine tuberculosis exhibits no greater heat resistance than does a suspension of active cultures of the bovine bacilli, made upon blood serum, when both are suspended in bouillon. Exposure to a temperature of 60°C. for twenty minutes was sufficient to destroy the bacilli in both. So long ago as 1887 Sternberg[15] found that tuberculosis sputum exposed to 60°C. for ten minutes was innocuous when injected into guinea pigs. Grancher and Ledoux-Lebard[16] note that avian tubercle bacilli are killed by exposure of twenty minutes at 60°C., but not of ten minutes, human bacilli were found dead after an exposure of ten minutes. Bonhoff[17] found a culture of the human tubercle bacilli in glycerinated broth was killed by an exposure of twenty minutes to 60°C., and more recently Rosenau[18] comes to a like conclusion. It must, however, be added that there have been certain contrary observations. Thus in 1888 Yersin[19] testing an old glycerine broth culture containing "sporulating bacilli" notes that these withstood being heated for ten minutes at a temperature of 60°C., after this exposure they continued to multiply and caused tuberculosis when

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14. Theobald Smith, loc. cit.

15. "Disinfection & Disinfectants". Report to the Amer.Public Health Assoc., Concord,N.H., 1888 : p.148.

16. Arch. de Med. Exper., Vol.IV : 1892 : p.1.

17. Hygienische Rundschau II : 1892 : p.1009.

18. U.S.Treasury Dept.,Hygienic Lab.Bulletin : No.42 : 1908.

19. Annales de l'Inst. de Pasteur, II : 1888 : p.60.

inoculated into rabbits. We are not told of the effects of heating to the same temperature for twenty minutes. deMan[20] employing the soft, almost cheesy matter, from the udders of tuberculous cows and heating this in sealed tubes found that this would stand an exposure of 60°C. for thirty and forty-five minutes. Two guinea pigs inoculated with material from these exposures became tuberculous, while after exposure for 60 minutes the guinea pigs remained well. He repeated and confirmed these results and found also that bacilli in sputum are rendered innocuous by exposure to 60°C. for sixty minutes. Here it will be seen that according to his results the time necessary for the destruction of the bacilli is considerably lengthened, and more recently Gavina[21] according to Wherry, is stated to have found that employing "sporulating" cultures he obtained tuberculosis in guinea pigs when these were inoculated with cultures heated for fifteen minutes at 96°C., but did not gain any results when he inoculated these animals with "non-spored" cultures which had been heated for ten minutes at 80°C.

It will be seen that so far as they go, our observations support and confirm those of Theobald Smith and Wherry, and fail to substantiate the observations of deMan and Gavina. What is obvious is that these spore-like bodies have no greater resistance to heat than that exhibited by many non-sporing bacteria.

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20. Arch. f. Hygiene XVIII, 1893 : p. 133

21. Ctbl. f. Bakt. Abt.1, Originale, 70 : 1913 : p.115.

Our wholly unexpected results with the Gonidia of Penicillium and Mucor indicate, however, that typical gonidial spores are possessed of no increased resistance to moist heat. It is thus still within a possibility that these bodies in the tubercle and diphtheroid bacilli are of the nature of gonidia, and as such are to be classed as spores; but if so, as bodies totally distinct in nature from the endospores of bacteria. I regret that I have obtained these results too late in the course of my observations to make a study of the thermal resistance of the gonidia-like bodies in the closely allied streptothrical forms. I would in conclusion suggest that there is need for a full inquiry into the properties of the gonidia of moulds and streptothrices. If, as here indicated, they are not heat resistant, to what extent are they resistant in other ways - to dessication for example, and to chemicals? Are we wrong in regarding them as resistant forms? Are they developed not so much in order to withstand adverse conditions as to be capable of easy transport and diffusion?

It is now my pleasant duty here to thank Prof. Lloyd and Prof. Derick of the Botanical Department, for their interest and assistance, and to acknowledge in all gratitude that whatever measure this paper possesses of scientific value is due to Prof. Adami, under whose supervision as head of the Bacteriological Department of the University, this work has been carried out.