development of the embryos took place during the first week, the drug apparently caused some permanent damage in that a significant number of them became moribund and died during the latter part of the incubation period. Because of this delayed toxicity of the maximal dosage, it is probable that some of the embryo deaths shown in Fig. 1 and 2 are attributable to the drug and not to the rickettsiae.

In addition to its activity against typhus rickettsiae, preliminary experiments have shown that paba also inhibits growth of the rickettsiae of Rocky Mountain spotted fever, but has no apparent effect on growth of psittacosis elementary bodies. Further experiments are now in progress on the mode of action of p-aminobenzoic acid.

*Conclusions.* I. p-Aminobenzoic acid inhibits the growth of typhus rickettsiae and prolongs the survival time of infected chick embryos. The inactivity of related compounds indicates that the inhibition is specific to paba. 2. The minimum amount of paba which will inhibit rickettsial growth is 0.4 mg per egg. In general, the inhibition is more complete with maximal dosages, which in the case of the 6-day chick embryo is 2-4 mg. These data suggest that relatively massive amounts of paba may have to be administered if beneficial effects are to be expected on human cases of typhus. 3. When treatment with paba is delayed 24, 48, and 72 hours beyond the time of infection, there is progressively less delay in the time of death and less reduction in the number of rickettsiae. However, good results are obtained with treatment as late as 48 hr after infection.

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## Penicillin as a Sporicidal Agent.

HAROLD R. CURRAN AND FRED R. EVANS. (Introduced by G. E. Holm.)

*From the Division of Dairy Research Laboratories, Bureau of Dairy Industry, Agricultural Research Administration, U. S. Department of Agriculture.*

Although Fleming's[1] first paper on penicillin furnished evidence of bactericidal as well as bacteriostatic activity, until 1941 attention was focused almost exclusively on the bacteriostatic or growth-inhibiting action of the drug.[2-4] Dawson *et al.*[5] reported that "Penicillin appears to be bactericidal against the pneumococcus *Streptococcus viridans,* staphylococcus and *Clostridium welchii, Cl. sporogenes,* and *Cl. septique*" (the latter three in the vegetative form). More recent reports have revealed strong bactericidal activity by penicillin against *Streptococcus hemolyticus,*[6-9] *S. viridans,*[10] pneumococci,[6,7] staphylococci,[7,11] and meningococci.[12]

Investigators[6,9,12,13] are essentially in agreement that penicillin activity is demonstrable only when the organisms are in an environment conducive to multiplication and it has been

1 Fleming, A., *Brit. J. Exp. Path.*, 1929, **10**, 226.

2 Chain, E., Florey, H. W., Gardner, A. D., Heatley, N. G., Jennings, M. A., On-Wing, J., and Sanders, A. G., *Lancet*, 1940, **2**, 226.

3 Abraham, E. P., Chain, E., Fletcher, C. M., Gardner, A. D., Heatley, N. G., Jennings, M. A., and Florey, H. W., *Lancet*, 1941, **2**, 177.

4 Reid, R. D., *J. Bact.*, 1935, **29**, 215.

5 Dawson, M. H., Hobby, G. L., Meyer, K., and Chaffee, E., *J. Clin. Invest.*, 1941, **20**, 434.

6 Hobby, G. L., Meyer, K., and Chaffee, E., PROC. SOC. EXP. BIOL. AND MED., 1942, **50**, 277.

7 Dawson, M. H., Hobby, G. L., Meyer, K., and Chaffee, E., *Ann. Int. Med.*, 1943, **19**, 707.

8 Robinson, H. L., *J. Pharm. and Exp. Therap.*, 1943, **77**, 70.

9 Hobby, G. L., and Dawson, M. H., PROC. SOC. EXP. BIOL. AND MED., 1944, **56**, 178.

10 Watson, R. H., PROC. SOC. EXP. BIOL. AND MED., 1944, **57**, 66.

11 Rantz, L. A., and Kirby, W. M., *J. Immun.*, 1944, **48**, 335.

12 Miller, C. P., and Foster, A. Z., PROC. SOC. EXP. BIOL. AND MED., 1944, **56**, 205.

shown[14] that the more active the multiplication the greater the effect of penicillin.

Since non-sporulating or vegetative bacteria seem to respond to penicillin only under narrowly circumscribed conditions of environment and physiologic activity, it is hardly surprising that bacterial spores have received no attention in penicillin studies.* Apparently it has been assumed that penicillin has no sporicidal activity. Observed facts, however, do not support this assumption. In this communication we shall show that spore suspensions may undergo a marked reduction in numbers when they are incubated for some hours with a relatively low concentration of penicillin.

We have previously shown† that in many aerobic species spores remain in the unchanged heat stable condition under favorable conditions for growth unless they have been previously heated for a few minutes at sublethal temperatures. The latter process termed heat activation promotes the change from the heat stable to the heat labile condition which is normally followed by vegetative development.

The action of penicillin upon bacterial spores in distilled water and in milk is shown in Table I. Washed spores (entirely free from vegetative cells except 1518) were added to and thoroughly mixed with the sterile test medium, then heated mildly for a few minutes to precondition them for germination as described in the preceding paragraph. Penicillin‡ (in a 5% glucose solution) was then added and allowed to act at a favorable temperature for several hours. At the end of the reaction period the residual penicillin was completely inactivated by means of a standardized sterile solution of penicillinase.§ The difference between the numbers of viable spores as determined by pour plates in the test and control suspensions before and after incubation is the measure of the sporicidal activity of the penicillin. Table I shows that with one exception penicillin effected a marked reduction in the number of viable spores when the periods of contact were 5 and 27 hours. The two strains of *B. subtilis* were the most susceptible. *B. cereus* apparently was unaffected. In both subtilis strains and *B. megatherium* the destruction of the spores was much greater in milk than water. This is contrary to usual experience wherein germicidal activity is almost universally diminished by the presence of organic matter. The greater activity of penicillin against the spores of *B. subtilis* and *B. megatherium* in milk seems to be accounted for by the data presented in Table II.

In this experiment a sample of milk was uniformly seeded with the spores of 2 strains of *B. subtilis*. Each inoculated sample was divided into 4 equal portions, 2 of these were heated to 95° for 15 min and 2 served as non-heated controls. One each of the heated and non-heated samples were treated with penicillin (5 u/ml), the remaining 2 served as non-penicillin controls. All samples were then incubated at 45° C. At the periods indicated samples were heated at 85° C for 10 min, treated with penicillinase, and plated. The data (Table II) show that the spores became heat labile at the same rate both with and without penicillin; also that the spores remained partially or wholly in the heat stable condition when they had not previously been heated to 95° for 15 min, whether they were exposed to penicillin or not.

The data in the last two columns (Table II) give the total number of viable organisms after the indicated periods of contact with penicillin. These figures compared with the numbers of heat stable spores (next two columns) show that the spores became heat labile much more rapidly than they were killed by penicillin. This lag between conversion of the spores to the heat labile state and evidence of penicillin activity suggests that in sensitive species a nutrient medium promotes penicillin action by permitting the spore to change to a more vulnerable condition at which time development

[13] Dawson, M. H., Hobby, G. L., Meyer, K., and Chaffee, E., *J. Clin. Invest.*, 1941, **20**, 434.

[14] Hobby, G. L., and Dawson, M. H., PROC. SOC. EXP. BIOL. AND MED., 1944, **56**, 181.

* Foster, J. W., and Woodruff, H. B. (*J. Biol. Chem.*, 1943, **148**, 723.) recommended the use of *B. subtilis* spores in the cup assay of penicillin, but with no suggestion that sporicidal activity might be involved.

† *J. Bact.*, in press.

‡ Penicillin sodium, kindly supplied by Chas. Pfizer and Co., Brooklyn, N. Y.

§ Obtained through the courtesy of the Schenley Research Institute, Lawrenceburg, Indiana.

TABLE I.
Sporicidal Activity of Penicillin 5u/ml in Water* and in Milk.*

| Culture | Medium | Temp. of reaction. °C | No. of viable spores per ml after contact with penicillin 0 hr × 1000 | 5 hr × 1000 | 27 hr × 1000 |
|---|---|---|---|---|---|
| *B. subtilis* (LB) | Water | 45 | 129 | 114 | 15.8 |
| | Milk | 45 | 261 | 1.6 | 0.1 |
| " " (6) | Water | 45 | 257 | 203 | 42 |
| | Milk | 45 | 256 | 1.6 | 0.04 |
| *B. megatherium* (696) | Water | 30 | 97 | 81 | 55 |
| | Milk | 30 | 141 | 32 | 8.7 |
| *B. cereus* (232) | Water | 30 | 67 | 71 | 43 |
| | Milk | 30 | 165 | 1,200[1] | Millions[1] |
| *B. stearothermophilus* (1518) | Water | 55 | 173 | 23 | 17 |
| | Milk | 55 | 166 | 24 | 11 |

* Suspensions heated at 95° C for 15 min before penicillin added.
[1] Spores and vegetative cells.
u = Oxford units.

TABLE II.
Changes in the Heat Stability of Heated and Non-heated Subtilis Spores When Incubated in Milk With and Without Penicillin. The Total Number of Organisms Surviving Penicillin Action When the Spores Were Previously Heated.

| Period of incubation | Number of heat stable spores per ml when treatment was: No penicillin: No heat before incubation—85° for 10 min after | | 95° for 15 min before incubation—85° for 10 min after | | Penicillin 5u/ml: No heat before incubation—85° for 10 min after | | 95° for 15 min before incubation—85° for 10 min after | | Total viable organisms per ml Penicillin 5u/ml: 95° for 15 min before incubation —no heat after | |
|---|---|---|---|---|---|---|---|---|---|---|
| | LB | 6 | LB | 6 | LB | 6 | LB | 6 | LB | 6 |
| min. | × 1000 | × 1000 | × 1000 | × 1000 | × 1000 | × 1000 | × 1000 | × 1000 | × 1000 | × 1000 |
| 0 | 39 | 46 | 40 | 46 | 44 | 42 | 44 | 42 | 44 | 42 |
| 5 | | | 39 | 34 | | | 40 | 33 | | |
| 10 | | | 31 | 19 | | | 28 | 20 | 43 | 42 |
| 15 | | | 16 | 15 | | | 17 | 17 | 44 | 43 |
| 30 | | | 2 | 6 | | | 2 | 7 | 44 | 42 |
| 60 | 43 | 26 | 0.2 | 2 | 41 | 26 | 0.2 | 2 | 36 | 35 |
| 120 | 39 | 19 | 0.1 | 1 | 42 | 18 | 0.2 | 0.3 | 9.7 | 10.2 |

LB and 6 = strains of *B. subtilis*.
Incubation temperature 45° C.

is apparently arrested and the organisms killed. The development of the spores to the heat labile stage is manifestly not necessary for all spores, since many are killed by penicillin in a non-nutrient medium (Table I). However, with sensitive species it may be easily demonstrated that preliminary mild heating of the spores in water increases their susceptibility to penicillin. Although the heat stability of these heated spores is unchanged, it is apparent that they have undergone certain germinative changes and represent therefore a stage of development which is intermediate between the untreated and heat labile stage. The relationship between susceptibility to penicillin and preliminary heating of spores is brought out in Table III. The effect of heat is pronounced with the Subtilis strains and with *B. megatherium*. The lower count after initial heating of the spores both of *B. megatherium* and *B. cereus* is evidence of killing by heat. With 1518, however, the initially lower count after heating is caused by the elimination of vegetative cells, which are difficult to exclude from the spore cultures of this organism. The check plates with 1518 in the presence of penicillin were inexplicably erratic.

Many, perhaps all, of these cultures produce penicillinase; hence to be effective, penicillin must prevent any considerable vegetative growth after germination of the spores. In suspensions of *B. cereus* spores, penicillin is

TABLE III.
Sporicidal Activity of Penicillin for Heated and Non-heated Spores.

| Culture | Penicillin 5u/ml added to milk—viable spores per ml | | | | | |
|---|---|---|---|---|---|---|
| | Non-heated spores | | | Heated spores[1] | | |
| | 0 hr × 1000 | 5 hr × 1000 | 27 hr × 1000 | 0 hr × 1000 | 5 hr × 1000 | 27 hr × 1000 |
| *B. subtilis* (LB) | 14.7 | 29.7 | 4.2 | 293 | 2.4 | 0.03 |
| | (293)[2] | (148) | (61) | (290) | (0.5) | (0.04) |
| " " (6) | 205 | 28.5 | 2.1 | 241 | 0.8 | 0.06 |
| | (241) | (19) | (2.7) | (241) | (0.4) | (0.1) |
| *B. megatherium* (696) | 231 | 86 | 23 | 116 | 25 | 6.2 |
| *B. cereus* (232) | 267 | 2,500[3] | Millions | 164 | 1,200[3] | Millions |
| *B. stearothermophilus* (1518) | 292 | 96 | 18.5 | 196 | 108 | 16.6 |

[1] 95°-15 min for LB, 6 and 1518; 85°-15 min for *B. megatherium* and *B. cereus*.
[2] Figures in parentheses show plate count after 95°-15 min.
[3] Both spores and vegetative cells.
Incubation temperature: *B. subtilis*, 45° C; *B. megatherium* and *B. cereus*, 30° C; *B. stearothermophilus*, 55° C.

clearly unable to prevent vegetation of germinated spores. Whether this is due to penicillinase production in the intermediate stages of germination or to other factors is not known. Increasing the concentration of penicillin or incubation of the suspensions containing it for longer periods, only slightly increased its sporicidal activity, indicating that even in susceptible species there exists a very small fraction of spores which are relatively resistant to penicillin.

The extraordinary effectiveness of penicillin in low concentration against certain types of spores, together with its non-toxic nature, suggests potential usefulness in non-medical fields, including that of food preservation. The possibility of employing penicillin in conjunction with mild heating merits consideration. Within a very limited sphere of observation, we have observed that a high degree of resistance to penicillin is correlated with a low degree of thermal resistance. Our observations upon the sporicidal activity of penicillin are being extended and will be reported later.

*Summary.* Under suitable conditions of incubation, penicillin in relatively low concentration is actively sporicidal against many aerobic species of bacteria. In sensitive species the sporicidal activity of penicillin is much greater in milk than in water and is usually enhanced by sublethal heating of the spores before treatment with penicillin.

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## Electron Shadow-Micrography of Virus Particles.*

ROBLEY C. WILLIAMS AND RALPH W. G. WYCKOFF.
*From the Department of Physics and the Virus Laboratory, School of Public Health, University of Michigan, Ann Arbor, Mich.*

The wave length of the light used with an optical microscope fixes the size of the smallest detail that it will resolve. For the shortest ultraviolet light it is practical to employ, this is in the neighborhood of 0.15 micron. The electron microscope, however, has such a great ultimate resolving power that, at least for the present, the smallest object it will reveal has a size determined by other factors. Photomicrographs produced with electrons are essentially shadow-graphs in which regions that are relatively light on the original negative correspond to regions in the sample which deflect (scatter) a larger percentage of the incident electrons,

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