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Dormancy of Spores of *Cl. Acetobutylicum* and *Cl. Pasteurianum*.

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The time required for the germination of anaerobic spores following single cell isolation is extremely variable, as may be seen from the records of 2 strictly anaerobic bacteria, *Cl. acetobutylicum* and *Cl. Pasteurianum*. Spores were picked with the modified Chambers micromanipulator and were immediately transferred to suitable culture media, beef-peptone-glucose agar, corn mash, milk, Hall's brain mash, or Speakman's synthetic medium. Sterile vaseline was layered onto the medium to maintain anaerobic conditions and to prevent evaporation during long incubation. From 100 isolations of *Cl. acetobutylicum* and 14 of *Cl. Pasteurianum* the 6 cultures as given in the table were obtained.

TABLE I.
The Germination Time of Single Anaerobic Spores.

| Culture | Medium | Time before germination |
|---------------------------|---------------------------|-------------------------|
| <i>Cl. acetobutylicum</i> | | days |
| 1. No. 105 | Beef-peptone-glucose-agar | 11 |
| 2. No. 105 | Milk | 117 |
| 3. No. 105 | Milk | 222 |
| 4. No. 70 | Brain mash | 19 |
| 5. No. 70 | Corn mash | 21 + (*) |
| <i>Cl. Pasteurianum</i> | | |
| 1. No. 5 | Synthetic | 72 |

(*) Germination between the 21st and 25th day.

Why should a spore lie dormant for 222 days or, for that matter, for 11 days before germination? Conditions of temperature, available food, and anaerobiosis were favorable during the entire period. As far as we know there was no change which might have acted as the final stimulus to germination. It was suggested that perhaps a spore, like a seed, needed a period of ripening after being set free from its mother sporangium. However, a review of the ages of spores when picked does not bear this out. No. 70 (4) with a dormancy of only 19 days came from a free spore in an agar colony 96 hours old while No. 105 (3) with the extreme dormancy of 222 days came from a stock culture in corn mash 1 year old.

Even the extreme germination time in the table is far shorter than the extreme recently reported by Dickson.¹ He found growth

¹ Dickson, E. C., PROC. SOC. EXP. BIOL. AND MED., 1928, xxv, 426.

in a thermal death point experiment on *Cl. botulinum* 72 months after heating. However, his *Cl. botulinum* spores may have been injured by heating while those of *Cl. acetobutylicum* and *Cl. Pasteurianum* picked directly from stock cultures should be of unimpaired vitality.

Why should some single spores, always a low percentage of those picked, germinate in the end. Shall we believe that the others were not viable when picked, died during incubation, or are still alive and dormant? We have no way of answering, but what we do know of dormancy in single spore isolations should make us very cautious about making definite statements that a culture is killed by a certain treatment or that a substance is free from anaerobic spores.

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Inhibition of Ovulation and Associated Histological Changes.*

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The idea that the corpus luteum is responsible for the inhibition of ovulation seems to have been first elaborated by Beard¹ and by Prenant.² The experimental proof of the inhibitory action of the corpus luteum has been attacked in many different ways. The two most general methods have been based on anatomical and physiological modifications of the reproductive tract, correlated with the presence or absence of a functional corpus luteum, and, changes produced by the injection of extracts of lutein tissue. Corner and Hurni³ reported negative results in an attempt to inhibit ovulation by injecting corpus luteum extracts in normal white rats. Loeb⁴ failed to obtain consistent positive results in the guinea pig, while Papanicolaou⁵ recently reported inhibition of ovulation in the same animal. Pearl and Surface⁶ were able to stop hens from laying by injections of water extracts of a dried commercial preparation.

* This work has been assisted by grants from the Committee for Research on Problems of Sex, of the National Research Council.

¹ Beard, J., Jena, 1897.

² Prenant, A., *Rev. Med. de l'Est.*, 1898, xxx, 385.

³ Corner, G. W., and Hurni, F. H., *Am. J. Phys.*, 1918, xlv, 483.

⁴ Loeb, L., *Biol. Bull.*, 1914, xxvii, 1.

⁵ Papanicolaou, G. N., *J. Am. Med. Assn.*, 1926, lxxxvi, 1422.

⁶ Pearl, R., and Surface, F. M., *J. Biol. Chem.*, 1914, xix, 236.