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Bacterial Endospore Formation in Media of Varying Biologic Value.

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Although the nature of the stimulus which causes a spore forming organism to enter the spore state is not known, a number of theories have been advanced, two of which are probably the most widely cited and most readily investigated experimentally. The first of these, advanced by Buchner,¹ holds that the inciting stimulus is to be found in a "local" exhaustion of nutrient material in the presence of an abundance of previously well nourished cell protoplasm. Turro² ascribed the cause of spore formation to the accumulation of metabolic by-products.

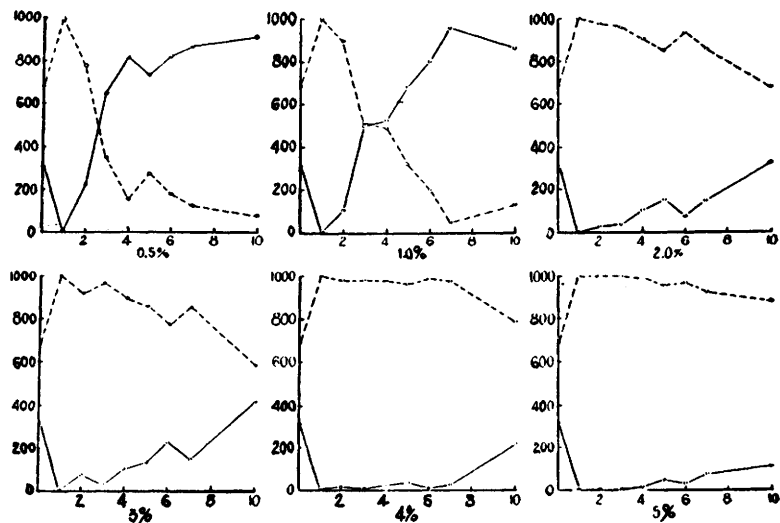
Some evidence on this subject is afforded by observations made on *B. subtilis* grown in peptone water of 0.5, 1, 2, 3, 4, and 5% peptone content. Inoculations were made in duplicate from an invigorated broth culture to 50 ml. flasks containing between 10 and 15 ml. of medium at a reaction of approximately pH 6.8. A count of 1000 cells of the inoculum gave 314 spores to 686 vegetative cells. Adequate exposure to oxygen was insured by the shallow layers of the medium. Incubation was at 37°C., the optimum for the strain of *B. subtilis* used. Smears were made from each flask at daily intervals for 7 days and again at 10 days, after which the observation was discontinued. From each smear, after staining, a total of 500 cells, including both spore and vegetative forms, was counted. There was no appreciable difference between duplicate flasks in either daily counts or total counts. In only 5 instances did the daily counts fail to agree within 15% of the average, and with the total counts the agreement in half the cases was within 2% or less and in no case exceeded 7%. As a matter of convenience the values obtained have been added together for plotting. The results are given in Table I and shown graphically in Fig. 1.

A consideration of these results at any time interval shows, with only a few minor discrepancies, that the percentage of spores varies inversely with the amount of peptone in the medium.

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¹ Buchner, H., *Sitzungsberichte der Math. physik Classe der Wissenschaften zu München*, 1880, X, 368. *Centralbl. Bakt.*, 1890, **8**, 1; 1896, I, **20**, 806.

² Turro, R., *Gaceta medica catalana*, 1891, 3 and 4. (Abst. in *Centralbl. Bakt.*, 1891, **10**, 91.



Abcissa—Days. Ordinate—Cells counted.
Broken line—Vegetative cells. Solid line—Spores.

FIG. 1.

TABLE I.

Day→	1		2		3		4		5		6		7		10	
% peptone	S	V	S	V	S	V	S	V	S	V	S	V	S	V	S	V
0.5	7	993	222	778	650	350	819	181	735	265	822	178	872	128	916	84
1	0	1000	109	891	497	503	520	480	680	320	796	204	956	44	866	134
2	0	1000	26	974	38	962	106	894	150	850	72	928	148	852	324	676
3	0	1000	80	920	33	967	104	896	133	867	227	773	144	856	419	581
4	0	1000	17	983	13	987	25	975	39	961	15	985	27	973	218	782
5	0	1000	0	1000	2	998	15	985	47	953	32	968	76	924	115	885

S = Spore. V = vegetative cell.

Determinations by direct microscopic count of the absolute number of spores and of vegetative cells in 1% and 5% peptone water after 10 days' incubation gave the values shown in Table II.

TABLE II.

	Spores x 10 ⁶	Vegetative cells x 10 ⁶	Total x 10 ⁶	Ratio—Spores to vegetative cells.
1% peptone water	952	497	1,449	1.94
5% peptone water	1,915	7,127	9,042	.27

If we assume, as would seem justifiable, that the ratio of spore to vegetative cell is of more significance in the physiology of spore formation than is the absolute number of spores formed, these re-

sults indicate that a depletion, or comparative paucity, of nutrient material is more important in promoting spore formation than is the accumulation of metabolites.

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Effects of Certain Broncho-Constricting Drugs on Intrapleural Pressure.*

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Report has previously been made¹ that following the administration to dogs of such typical broncho-dilating drugs as epinephrin and atropine, there is a definite increase in intrapleural pressure, *i. e.*, intrapleural pressure becomes more positive than is normally the case. This was interpreted as being due to lessened suction on expansion of the chest because of lessened resistance to the movement of air in and out of the lungs as a result of broncho-dilatation. It naturally became of interest to determine by direct observation whether or not the corollary is also true, *viz.*, that broncho-constriction is followed by the development of more negative intrapleural pressure than is normally present.

We employed the same dogs as had been used in our former experiments, and we had sufficient observations on normal intrapleural pressures in these animals to enable us to judge whether or not changes following drug administration were beyond normal diurnal variation. The technique used was the same as that previously described.¹

Following the subcutaneous injections of solutions of pilocarpine nitrate and physostigmine (eserine) salicylate, we found uniformly in 6 experiments with each drug that intrapleural pressure definitely became less, *i. e.*, more negative. The experiments were made on 3 dogs, with intervals of a week or more between experiments. We were unable to draw definite conclusions following the administration of histamine or of arecoline because of marked disturbances in character of respiration and in the condition of the animal. Quantitative data from sample experiments may be found in Table I.

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¹ Brill, S., and Leake, C. D., *PROC. SOC. EXP. BIOL. AND MED.*, 1930, **27**, 518.